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**WO 01/62968 A2**

(54) Title: **MUTANT NUCLEIC BINDING ENZYMES AND USE THEREOF IN DIAGNOSTIC, DETECTION AND PURIFICATION METHODS**

(57) Abstract: Methods for detecting, localizing and removing abnormal base-pairing in a nucleic acid duplex are provided. These methods can be used for prognosis and diagnosis of diseases, disorders, pathogenic infections and nucleic acid polymorphisms. Combinations, kits and articles of manufacture for use in these methods are also provided.

5

**MUTANT NUCLEIC BINDING ENZYMES AND USE THEREOF IN  
DIAGNOSTIC, DETECTION AND PURIFICATION METHODS**

10

**RELATED APPLICATIONS**

15

This application is related to U.S. application Serial

No. 09/347,878 to Chong-Shen Yuan, filed July 6, 1999, entitled

"COMPOSITIONS AND METHODS FOR ASSAYING ANALYTES" and U.S.

application Serial No. 09/457,205 to Chong-Shen Yuan, filed

20 December 6, 1999, entitled "COMPOSITIONS AND METHODS FOR

ASSAYING ANALYTES." U.S. application Serial No. 09/457,205 is a

continuation-in-part application of U.S. Patent Application Serial No.

09/347,878, filed July 6, 1999, now pending. The contents of each of these applications is incorporated herein in its entirety.

### **FIELD OF THE INVENTION**

5       Methods for detecting nucleic acids that contain any abnormal base-pairing in a nucleic acid duplex are provided. The methods are particularly useful for prognosis and diagnosis of diseases, disorders and pathogenic infections and for detection of nucleic acid polymorphisms. Also provided are mutant nucleic acid binding enzymes, particularly repair 10 enzymes, that retain binding specificity and affinity, but lack catalytic activity. Combinations, kits and articles of manufacture that contain these mutant enzymes are also provided.

### **BACKGROUND OF THE INVENTION**

15       In the wake of the human genome project, future medical practice will use more and more human genetic information for disease prognosis, diagnosis and prevention. The need for rapid and accurate methods of genetic variation detection are escalating. It is these nucleic acid mutation detection technologies that will ultimately help to reveal the

relation between human genetic makeup and diseases. Although methods are available for detecting DNA mutations/polymorphisms, none is suitable for use in a high throughput format for detecting large numbers of mutations/polymorphisms simultaneously in a single assay format.

5        This lack of suitability derives from the requisite use of specific probes for detecting mutations in the target nucleic acids. For example, PCR-restriction fragment length polymorphism (PCR-RFLP) (see, e.g., Bashiruddin, *Methods Mol. Biol.*, 104:167-78 (1998); Hyland et al., *Transfus. Med. Rev.*, 9(4):289-301 (1995); Gasser and Chilton, *Acta 10 Trop.*, 59(1):31-40 (1995); and Pourzand and Cerutti, *Mutat. Res.*, 288(1):113-21 (1993)), not only requires the design of target-specific probes, but also involves a gel-electrophoresis step to analyze the DNA digestion patterns in comparison with the wild type gene. It is a time consuming and expensive procedure. Similar problem exists with other 15 methods such as single-strand conformation polymorphism (PCR-SSCP) detection, which also requires specific probes and gel-electrophoresis (Hayashi and Yandell, *Hum. Mutat.*, 2(5):338-46 (1993); Hayashi, *Genet. Anal. Tech. Appl.*, 9(3):73-9 (1992); and Hayashi, *PCR Methods Appl.*, 1(1):34-8 (1991)). Methods, such as the Invader™ assay (Third Wave

Technologies, Inc.) for detection of polymorphism based on the use of Cleavase enzymes to cleave a complex formed by hybridization of overlapping oligonucleotide probes (Marshall et al., *J. Clin. Microbiol.*, 35(12):3156-62 (1997)) eliminates the gel-electrophoresis step, but the 5 method requires more probes specific for the genes to be tested.

Moreover, the Invader™ assay method works only when the exact mutation and mutation position are known. Therefore, it is difficult to automate this method for detecting large number of genes in a single format.

10 Therefore, a need to develop nucleic acid detection and mapping methods amenable to high throughput formats. Thus, it is an object herein to provide a nucleic acid mutation detecting method that requires neither specific probes nor gel-electrophoresis. It is another object herein to provide a nucleic acid mutation detecting method that is amendable to 15 automation for simultaneous detection of large numbers of nucleic acid mutations.

**SUMMARY**

Provided herein are nucleic acid mutation detecting methods that meet the above-noted objectives. These methods have wide application in various areas such as prognosis and diagnosis of diseases, disorders or pathological infections, and selectively binding, such as for removal or purification, nucleic acid duplexes that include abnormal base-pairings in a population of nucleic acid duplexes.

The nucleic acid mutation detecting methods provided herein use mutant nucleic acid binding enzymes, such as mutant repair enzymes, and other enzymes that specifically bind to abnormal base pairs, such as base-pair mismatch, a base insertion, a base deletion and a pyrimidine dimer. The mutant enzymes substantially retain the specific binding affinities for abnormal base-pairings of the wild-type enzymes but have reduced or lack the catalytic activities. The mutant enzymes thus act like an antibody (herein designated a pseudo-antibody) and specifically bind to abnormal base-pairings in a duplex. The mutant enzymes are enzymes, such as repair enzymes, particularly DNA repair enzymes, that typically bind to abnormally matched base pairs, such as base-pair mismatches, base insertions, a base deletions and pyrimidine dimers, and then catalytically repair the duplex. Methods of detection, diagnosis and other methods that rely on the affinity of the mutant enzymes for duplexes with abnormal base pairings, such as mismatches, are provided.

Among the methods provided, are methods for identifying and quantifying mutations. These methods are based upon the specificity of the mutant enzyme for a particularly abnormal base pairing. Hybridizing perfectly matched nucleic acid strands forms a nucleic acid duplex without any abnormal base-pairings and hybridizing imperfectly matched nucleic acid strands forms a nucleic acid duplex with one or more abnormal base-pairings. By contacting the formed nucleic acid duplex with one or more mutant repair enzyme(s), the duplex containing

abnormal base-pairing(s) binds to the mutant repair enzyme. Detection and quantitation of the complex formed between the nucleic acid duplex with the one or more abnormal base-pairings and the mutant DNA repair enzyme leads to identification and quantitation of nucleic acid mutations.

- 5        Hence, provided herein is a method for detecting abnormal base-pairing in a nucleic acid duplex by contacting a nucleic acid duplex having or suspected of having an abnormal base-pairing with a mutant DNA repair enzyme or complex thereof that has binding affinity for the abnormal base-pairing in the duplex but has attenuated catalytic activity;
- 10      and then detecting binding between the nucleic acid duplex and the mutant DNA repair enzyme or complex thereof. The amount of mutant enzyme bound is used to assess the presence or quantity of the abnormal base-pairing in the duplex.

The nucleic acid duplex that is assayed includes DNA:DNA,  
15      DNA:RNA and RNA:RNA duplexes. Preferably, the nucleic acid duplex to be assayed is a DNA:DNA duplex.

The abnormal base-pairing that is detected can be, for example, a base-pair mismatch, a base insertion, a base deletion or a pyrimidine dimer. Among the preferred uses of the mutant enzymes is for detection  
20      of a single base-pair mismatch. Such mismatches include, but are not limited to, A:A, A:C, A:G, C:C, C:T, G:G, G:T, T:T, C:U, G:U, T:U, U:U, 5-formyluracil (fU):G, 7,8-dihydro-8-oxo-guanine (8-oxoG):C, 8-oxoG:A and any combination thereof. Also preferably, the base insertion or base deletion to be detected is a single base insertion or deletion. For  
25      example, the base insertion or base deletion resulting in a single-stranded loop containing about 1-5 bases or a loop containing more than 5 bases can be detected.

Mutant DNA repair enzyme or complexes thereof that can be used in these methods include a mutant of any nucleic acid repair enzyme (or  
30      enzyme complex) as long as the mutant retains its ability to specifically bind to the nucleic acid that the wild-type repairs, but lacks substantial

catalytic activity. Enzymatic systems capable of recognition and correction of base pairing errors within the DNA helix have been demonstrated in bacteria, fungi and mammalian cells. Enzymes from any such system is contemplated herein. The enzyme can be mutagenized

5 using standard procedures, either directed mutagenesis if the catalytic site is known, or systematic mutagenesis to empirically identify suitable mutations. The resulting enzymes are selected for their ability to bind to abnormally, such as mismatched, paired DNA but to not effect repair or catalytic activity. Exemplary enzymes include, but are not limited to,

10 a mutant mutH, a mutant mutL, a mutant mutM, a mutant mutS, a mutant mutY; a mutant uvrD, a mutant dam, a mutant thymidine DNA glycosylase (TDG), a mutant mismatch-specific DNA glycosylase (MUG), a mutant AlkA, a mutant MLH1, a mutant MSH2, a mutant MSH3, a mutant MSH6, a mutant Exonuclease I, a mutant T4 endonuclease V, a

15 mutant FEN1 (RAD27), a mutant DNA polymerase  $\delta$ , a mutant DNA polymerase  $\epsilon$ , a mutant RPA, a mutant PCNA, a mutant RFC, a mutant Exonuclease V, a mutant DNA polymerase III holoenzyme, a mutant DNA helicase, a mutant RecJ exonuclease, a cleavase and combinations thereof (see below for definitions of each enzyme).

20 Also provided herein are methods for detecting a mutation in a nucleic acid. The methods are performed by hybridizing a strand of a nucleic acid having or suspected of having a mutation with a complementary strand of a wild-type nucleic acid, whereby if a mutation is present, the resulting duplex contains an abnormal base-pairing;

25 contacting the resulting duplex with a mutant nucleic acid repair enzyme or complex thereof; and detecting binding between the nucleic acid duplex and the mutant nucleic acid repair enzyme or complex thereof. The amount of enzyme bound is used to assess the presence or quantity of the mutation. Depending upon the mutant enzyme selected, the

30 identity of the mismatch may be determined as well. The nucleic acid strand to be tested and the complementary wild-type nucleic acid strand,

**MISSING AT THE TIME OF PUBLICATION**

sclerosis (ALS), Angelman syndrome (AS), Charcot-Marie-tooth disease (CMT), epilepsy, tremor, fragile X syndrome, Friedreich's ataxia (FRDA), Huntington disease (HD), Niemann-Pick, Parkinson disease, Prader-Willi syndrome (PWS), spinocerebellar atrophy and Williams syndrome.

5 Examples of signal diseases and disorders include, but are not limited to, ataxia telangiectasia (A-T), male pattern baldness, acne, hirsutism, Cockayne syndrome, glaucoma, mammals with abnormal secondary sexual characteristics, tuberous sclerosis, Waardenburg syndrome (WS) and Werner syndrome (WRN).

10 Exemplary transporter diseases and disorders include, but are not limited to, cystic fibrosis (CF), diastrophic dysplasia (DTD), long-QT syndrome (LQTS), Menkes' syndrome, pendred syndrome, adult polycystic kidney disease (APKD), Wilson's disease and Zellweger syndrome.

15 Other examples of the diseases and disorders that can be detected by the present methods include, but are not limited to, a disease or disorder associated with an androgen receptor mutation, tetrahydrobiopterin deficiencies, X-Linked agammaglobulinemia, a disease or disorder associated with a factor VII mutation, anemia, a disease or

20 disorder associated with a glucose-6-phosphate mutation, the glycogen storage disease type II (Pompe Disease), hemophilia A, a disease or disorder associated with a hexosaminidase A mutation, a disease or disorder associated with a human type I or type III collagen mutation, a disease or disorder associated with a rhodopsin or RDS mutation, a

25 disease or disorder associated with a L1CAM mutation, a disease or disorder associated with a LDL receptor mutation, a disease or disorder associated with an ornithine transcarbamylase mutation, a disease or disorder associated with a PAX6 mutation and a disease or disorder associated with a von Willebrand factor mutation.

30 The methods herein can also be used to detect infections and pathogens associated therewith. Such infection include, but are not

limited to, infections caused by a virus, a eubacteria, an archaeabacteria and a eukaryotic pathogen. The infections can be caused by a mutant strain of a virus, an eubacteria, an archaeabacteria or an eukaryotic pathogen.

- 5        Exemplary viruses include, but are not limited to, a Delta virus, a dsDNA virus, a retroid virus, a satellite virus, a ssDNA virus, a ssRNA negative-strand virus, ssRNA positive-strand virus (no DNA stage) and a bacteriophage. Eubacteria include, but are not limited to, a green bacteria, a flavobacteria, a spirochetes, a purple bacteria, a gram-positive bacteria, a gram-negative bacteria, a cynobacteria, a deinococci and a thermotogale. Archaeabacteria include, but are not limited to, an extreme halophile, a methanogen and an extreme thermophile. Eukaryotic pathogens include, but are not limited to, a fungi such as a yeast, a ciliate, a cellular slime mode, a flagellate and a microsporidia.
- 10
- 15        In the above methods for detecting mutations, the hybridization between the strand of a nucleic acid having or suspected of having a mutation and the complementary strand of a wild-type nucleic acid can be facilitated by a recombinase. Recombinase, include, but are not limited to, Cre recombinase, RAG-1 V(D)J recombinase, Endonuclease II of coliphage T4 and Flp recombinase.
- 20

Also provided herein are methods for detecting polymorphisms, including single nucleotide polymorphisms (SNPs) at a gene locus or loci. The methods include hybridizing a target strand of a nucleic acid molecule that includes the locus to be tested with a complementary reference strand of a nucleic acid that has a known allele of the locus. Allelic identity between the target and the reference strand results in the formation of a nucleic acid duplex without an abnormal base-pairing, and allelic difference between the target and the reference strands results in the formation of a nucleic acid duplex with an abnormal base-pairing.

- 25
- 30        The resulting nucleic acid duplex formed is contacted with a mutant nucleic acid repair enzyme or complex thereof that has binding affinity for

the abnormal base-pairing in the duplex but has attenuated catalytic activity. Binding between the nucleic acid duplex and the mutant DNA repair enzyme or complex thereof is detected. The presence of a polymorphism is then assessed. Any polymorphism may be detected by 5 these methods, and include, but are not limited to, a variable nucleotide type polymorphism ("VNTR"), a single nucleotide polymorphism (SNP), preferably a human genome SNP.

In the above methods for detecting polymorphisms, the hybridization between the target strand of a nucleic acid comprising a 10 locus to be tested and the complementary reference strand of a nucleic acid comprising a known allele of the locus can be facilitated by a recombinase. Recombinases include, but are not limited to, Cre recombinase, RAG-1 V(D)J recombinase, Endonuclease II of coliphage T4 or Flp recombinase.

15 Methods for selecting, purifying or removing a nucleic acid duplex containing one or more abnormal base-pairings in a population of nucleic acid duplexes are also provided. These methods are performed by contacting a population of nucleic acid duplexes having or suspected of including an abnormal base-pairing with a mutant DNA repair enzyme or 20 complex thereof, where the mutant DNA repair enzyme or complex thereof has binding affinity for the abnormal base-pairing in the duplex but has attenuated catalytic activity, whereby the nucleic acid duplex containing one or more abnormal base-pairing binds to the mutant DNA repair enzyme or complex thereof to form a binding complex. The 25 resulting complex can be removed from the population. The mutant enzyme can be presented and introduced into the population on a solid support, whereby duplexes in the population that contain an abnormal base pairing to which the mutant enzyme binds will bind to the enzyme on the solid support. In a specific embodiment, the population of nucleic acid duplexes contains DNA:DNA, DNA:RNA or RNA:RNA duplexes. The 30 abnormal base-pairing to be removed includes a base-pair mismatch, a

base insertion, a base deletion or a pyrimidine dimer. Preferably, the base-pair mismatch to be removed is a single base-pair mismatch.

The population of nucleic acid duplexes is produced by an amplification, such as by a polymerase chain reaction or a reaction using  
5 reverse transcription and subsequent DNA amplification of one or more expressed RNA sequences.

Further provided herein are methods for detecting and localizing an abnormal base-pairing in a nucleic acid duplex. These methods are performed by contacting a nucleic acid duplex having or suspected of  
10 having an abnormal base-pairing with a mutant DNA repair enzyme or complex thereof, where the mutant DNA repair enzyme or complex thereof has binding affinity for the abnormal base-pairing in the duplex but has attenuated catalytic activity, whereby the nucleic acid duplex containing an abnormal base-pairing binds to the mutant DNA repair  
15 enzyme or complex thereof to form a binding complex; subjecting the nucleic acid duplex to hydrolysis with an exonuclease under conditions such that the binding complex blocks hydrolysis; and then determining the location within the nucleic acid duplex protected from the hydrolysis, thereby detecting and localizing the abnormal base-pairing in the nucleic  
20 acid duplex. In a specific embodiment, the nucleic acid duplex to be assayed is a DNA:DNA, a DNA:RNA or a RNA:RNA duplex. Preferably, the nucleic acid duplex to be assayed is a DNA:DNA duplex. The abnormal base-pairing to be detected and localized is a base-pair mismatch, a base insertion, a base deletion or a pyrimidine dimer.  
25 Preferably, the base-pair mismatch to be detected and localized is a single base-pair mismatch. Exemplary exonucleases, include, but are not limited to, BAL-31 exonuclease, exonuclease III, Mung Bean exonuclease and Lambda exonuclease.

In the above methods for detecting abnormal base-pairings,  
30 mutations, and polymorphisms, and the methods for localizing and removing abnormal base-pairings, the mutant DNA repair enzyme or

complex thereof can be labelled. Preferably, the mutant DNA repair enzyme or complex thereof used therein is labelled, with a detectable label, such as biotin, a bioluminescence generating reagent, such as a luciferin or luciferase, a fluorescence label or a radiolabel, and the binding

- 5 between the abnormal base-pairing and the labelled mutant DNA repair enzyme or complex thereof is detected, such as with a streptavidin labeled enzyme, generation of bioluminescence by contacting with luciferin or luciferase, or detection of the fluorescence or bound radioactivity. Labeled enzymes, include but are not limited to, a
- 10 peroxidase, a urease, an alkaline phosphatase, a luciferase and a glutathione S-transferase. The mutant repair enzyme may also be prepared as a conjugate, such as a chemical conjugate or fusion protein, with a detectable label or tag or enzyme or enzyme substrate.

In the above methods for detecting abnormal base-pairings,

- 15 mutations, and polymorphisms, and the methods for localizing and removing abnormal base-pairings, the target nucleic acid strand to be assayed, the reference nucleic acid strand, the target nucleic acid duplex to be assayed, the nucleic acid duplex formed via hybridization of the target strand and the reference strand, or the mutant DNA repair enzyme
- 20 or complex thereof can be immobilized on the surface of a support, either directly or indirectly, such as via a linker. Preferably, the support used is an insoluble support such as a silicon chip. Support geometries, include, but are not limited to, beads, pellets, disks, capillaries, hollow fibers, needles, solid fibers, random shapes, thin films, membranes and
- 25 chips. Also more preferably, the nucleic acid strand, the nucleic acid duplex or the mutant DNA repair enzyme or complex thereof is immobilized in an array or a well format on the surface of a support. Immobilization can be effected via covalent, ionic or other interactions, and can be direct or via a suitable linking moiety, such as
- 30 heterobifunctional linker.

In the above methods, one sample can be assayed at one time, but

preferably, the assays are performed in high-throughput format where a plurality of samples are assayed simultaneously.

In the above methods, the target nucleic acid strand or target nucleic acid duplex can be synthesized or derived from a natural source.

- 5 In a specific embodiment, the target strand of a nucleic acid or the target nucleic acid duplex is isolated from a natural sample, e.g., a biosample. Preferably, the sample is a body fluid or a biological tissue. More preferably, the body fluid is urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, mucus or amniotic fluid. Also  
10 more preferably, the biological tissue is connective tissue, epithelium tissue, muscle tissue, nerve tissue, organs, tumors, lymph nodes, arteries and individual cell(s).

Mutant enzymes that substantially retain binding affinity and specificity, but that have reduced catalytic activity are also provided.

- 15 Compositions containing the mutant enzymes, kits and articles of manufacture containing the mutant enzymes are also provided. In particular a mutant nucleic repair enzyme that retains binding affinity for abnormal base pairs in a nucleic acid duplex, but has reduced catalytic activity compared to wild type, such that the mutant enzyme  
20 quantitatively retain a duplex on a solid support, with a  $K_a$  of at least about  $10^7$ , more preferably  $10^8$ , most preferably  $10^9$  M or higher.

The mutant enzymes include a mutant mutL is an *E.Coli* mutant mutL having a mutation selected from E29K, E32K, A37T, D58N, G60S, G93D, R95C, G96S, G96D, S112L, A16T, A16V, P305L, H308Y,  
25 G238D, S106F and A271V; a mutant MLH1 that is a human mutant MLH1 having a mutation selected from among of P28L, M35R, S44F, G67R, I68N, I107R, T117R, T117M, R265H, V185G and G224D; a mutant mutS that has a mutation in its catalytic site, dimerization site, mutL interaction site or combinations thereof; a mutM that has a  
30 mutation in its catalytic site, mutY interaction site or a combination thereof, including an *E.Coli* mutant mutM having a K57G or K57R

mutation; a mutant mutY that has a mutation in its catalytic site, mutM interaction site or a combination thereof, in an *E.Coli* mutant mutY having a mutation selected from among E37S, V45N, G116D, D138N and K142A; or is a mutant uvrD that has a mutation in its catalytic site, ATP binding site or a combination thereof, including an *E.Coli* mutant uvrD having a mutation selected from among K35M, D220NE221Q, E221Q and Q251E; a mutant MSH2 that has a mutation in its catalytic site, ATP binding site, ATPase site or a combination thereof, including an *S.cerevisiae* mutant MSH2 having a G693D or a G855D mutation and a 5 human mutant MSH2 having a fragment encoding 195 amino acids within the C-terminal domain of hMSH-2 or having a K675R mutation; a mutant MSH6 that has a mutation in its catalytic site, ATP binding site, ATPase site or any combination thereof, including a human mutant MSH6 having a K1140R mutation, a complex of a human mutant MSH2 having a 10 K675R mutation and a human mutant MSH6 having a K1140R mutation; and a mutant T4 endonuclease V that has a E23Q mutation.

15 Solid supports, such as silicon chips, containing one or a plurality of the same or of different mutant enzymes conjugated, either directly or indirectly, thereto, are also provided.

20 Kits and articles of manufacture for detecting abnormal base-pairings, mutations, polymorphisms, and for localizing and/or removing abnormal base-pairings are provided herein. The combinations, kits and articles of manufacture typically include one or more of the mutant enzymes, which may be in a composition or provided in an array or in 25 combination with a support with linked nucleic acids.

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  - 3) DNase I footprint analysis
  - 4) Screening a *λgt11* expression library with recognition-site DNA
  - 10 5) Rapid separation of protein-bound DNA from free DNA
- f. Lipid binding moieties
- g. Polysaccharide binding moieties
- 15 h. Metal binding moieties
- i. Other facilitating agents
  - 1) Peroxidase
  - 2) urease
  - 3) Alkaline phosphatase
  - 20 4) Luciferase
  - 5) Glutathione S-transferase
  - 6) Defense proteins
  - 7) Fluorescent moieties

H. IMMOBILIZATION OF MUTANT ENZYMES AND NUCLEIC ACIDS

- 25 1. Immobilization of the mutant enzymes
- 2. Immobilization of nucleic acids
- I. HIGH-THROUGHPUT ASSAY FORMAT
- 30 1. High-throughput assay instrumentation and capabilities
- 2. Detection technologies
  - a. Radiochemical methods
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- 1) Colorimetry and luminescence
- 2) Resonance energy transfer
- 3) Time-resolved fluorescence
- 4) Cell-based fluorescence assays

5                   5) Fluorescence polarization

- 6) Fluorescence correlation spectroscopy

3. Miniaturization

J. SAMPLE COLLECTION

K. COMBINATIONS, KITS AND ARTICLES OF MANUFACTURE

10 A. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, applications, published applications and other publications and sequences from GenBank and other data bases referred to herein are incorporated by reference in their entirety.

As used herein, "base-pairing" refers to the specific hydrogen bonding between purines and pyrimidines in double-stranded nucleic acids. In DNA, the pairs are adenine (A) and thymine (T), and guanine (G) and cytosine (C), while in RNA they are adenine (A) and uracil (U), and guanine (G) and cytosine (C). Base-pairing leads to the formation of a nucleic acid double helix from two complementary single strands.

As used herein, "nucleic acid duplex having abnormal base-pairing" refers to a nucleic acid duplex wherein there exists base-pair mismatch, i.e., any base-pairing other than any of the normal A:T(U) and C:G pairs, a single-stranded loop region due to the addition of extra-nucleotide(s) in one strand and/or deletion of nucleotide(s) in the complementary strand, or a combination thereof. Non-limiting examples of base-pair mismatch include A:A, A:C, A:G, C:C, C:T, G:G, G:T, T:T, C:U, G:U, T:U, U:U, 5-formyluracil (fU):G, 7,8-dihydro-8-oxo-guanine (8-oxoG):C, 8-oxoG:A.

As used herein, "enzyme" refers to a protein specialized to catalyze

or promote a specific metabolic reaction. Generally, enzymes are catalysts, but for purposes herein, such "enzymes" include those that would be modified during a reaction. Since the enzymes are modified to eliminate or substantially eliminate catalytic activity, they will not be so-modified during a reaction.

As used herein, "DNA repair" refers to a process wherein the sites of mutations in DNA (DNA:DNA duplexes, DNA:RNA and, for purposes herein, also RNA:RNA duplexes) are recognized by a nuclease that excises the damaged or mutated region from the nucleic acid; and then further enzymes or enzymatic activities synthesize a replacement portion of a strand(s) so that the original sequence is preserved.

As used herein, "DNA repair enzyme" refers to an enzyme that corrects errors in nucleic acid structure and sequence, i.e., recognizes, binds and corrects abnormal base-pairing in a nucleic acid duplex. DNA repair enzyme functions to protect genetic information against environmental damage and replication errors. Examples of DNA repair enzyme include mutH, mutL, mutM, mutS, mutY, uvrD, dam, thymidine DNA glycosylase (TDG), mismatch-specific DNA glycosylase (MUG), AlkA, MLH1, MSH2, MSH3, MSH6, Exonuclease I, T4 endonuclease V, FEN1 (RAD27), DNA polymerase  $\delta$ , DNA polymerase  $\epsilon$ , RPA, PCNA and RFC. It is intended that DNA repair enzymes encompasses enzymes with conservative amino acid substitutions that do not substantially alter repair activity. Suitable conservative substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson *et al.* *Molecular Biology of the Gene*, 4th Edition, 1987, The Bejacmin/Cummings Pub. co., p.224).

Such substitutions are preferably made in accordance with those set forth in TABLE 1 as follows:

TABLE 1

	Original residue	Conservative substitution
5	Ala (A)	Gly; Ser
	Arg (R)	Lys
	Asn (N)	Gln; His
	Cys (C)	Ser
	Gln (Q)	Asn
	Glu (E)	Asp
10	Gly (G)	Ala; Pro
	His (H)	Asn; Gln
	Ile (I)	Leu; Val
	Leu (L)	Ile; Val
	Lys (K)	Arg; Gln; Glu
	Met (M)	Leu; Tyr; Ile
15	Phe (F)	Met; Leu; Tyr
	Ser (S)	Thr
	Thr (T)	Ser
	Trp (W)	Tyr
	Tyr (Y)	Trp; Phe
	Val (V)	Ile; Leu

Other substitutions are also permissible and may be determined empirically or in accord with known conservative substitutions.

As used herein, the "amino acids," which occur in the various amino acid sequences appearing herein, are identified according to their well-known, three-letter or one-letter abbreviations. The nucleotides, which occur in the various DNA fragments, are designated with the standard single-letter designations used routinely in the art.

As used herein, "a mutant DNA repair enzyme" (used interchangeably with "abnormal base-pairing trapping enzyme") refers to a mutant form of an enzyme that can repair errors in duplexes. The mutant, however, has binding affinity for the abnormal base-pairing in a nucleic acid duplex but lacks the catalytic activity whereby the abnormal pairing is excised. The mutant form of the repair enzyme that retains sufficient binding affinity for the abnormal base-pairing to be detected in the process or method, particularly assay, of interest. Typically this is at least about 10%, preferably at least about 50% binding affinity for the abnormal base-pairing, compared to its wildtype counterpart. Preferably, such mutant DNA repair enzyme retains 60%, 70%, 80%, 90%, 100% binding affinity for the abnormal base-pairing compared to its wildtype

counterpart, or has a higher binding affinity than its wildtype counterpart. Such mutant DNA repair enzyme is herein referred to as an "abnormal base-pairing trapping enzyme", *i.e.*, a molecule that specifically binds to a selected abnormal base-pairing, but does not catalyze conversion thereof.

5 The mutant enzyme possess substantially reduced such that the binding of the enzyme to the duplex can be detected. This is typically no more than about 50%, preferably no more than 20%, more preferably no more than about 10%, of the wild-type catalytic activity.

As used herein the term "assessing" is intended to include

10 quantitative and qualitative determination in the sense of obtaining an absolute value for the amount or concentration of the abnormal base-pairing present in the sample, and also of obtaining an index, ratio, percentage, visual or other value indicative of the level of abnormal base-pairing in the sample. Assessment may be direct or indirect and the

15 chemical species actually detected need not of course be the abnormal base-pairing itself but may for example be a derivative thereof or some further substance.

As used herein, "attenuated catalytic activity" refers to a mutant DNA repair enzyme that retains sufficiently reduced catalytic activity to

20 be useful as a "pseudo-antibody", *i.e.*, a molecule used in place of an antibody in immunoassay formats. The precise reduction in catalytic activity for use in the assays can be empirically determined for each assay. Typically, the enzyme will retain less than about 50% of one of its catalytic activities or less than 50% of its overall catalytic activities

25 compared to its wildtype counterpart. Preferably, a mutant DNA repair enzyme retains less than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of one of its catalytic activities or its overall catalytic activities compared to its wildtype counterpart. More preferably, a mutant DNA repair enzyme lacks detectable level of one of its catalytic activities or its overall

30 catalytic activities compared to its wildtype counterpart. In instances in which catalytic activity is retained and/or a further reduction thereof is

desired, the contacting step can be effected in the presence of a catalysis inhibitor. Such inhibitors, include, but are not limited to, heavy metals, chelators or other agents that bind to a co-factor required for catalysis, but not for binding, and other such agents.

5        As used herein, "mutH" refers to a procaryotic latent endonuclease that incises the transiently unmethylated strands of hemimethylated 5'-GATC-3' sequences. It is intended to encompass mutH with conservative amino acid substitutions that do not substantially alter its activity.

10      As used herein, "mutS" refers to a procaryotic DNA-mismatch binding protein that can bind to a variety of mispaired bases and small (1-5 bases) single-stranded loops. It is intended to encompass mutS with conservative amino acid substitutions that do not substantially alter its activity.

15      As used herein, "mutL" refers to a procaryotic protein that couples abnormal base-pairing recognition by mutS to mutH incision at the 5'-GATC-3' sequences in an ATP-dependent manner. It is intended to encompass mutL with conservative amino acid substitutions that do not substantially alter its activity.

20      As used herein, "uvrD" refers to a procaryotic DNA helicase II that unwinds DNA in an ATP-dependent manner. It is intended to encompass uvrD with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "dam" refers to a procaryotic adenine

25      methyltransferases that plays a role in coordinating DNA replication initiation, DNA mismatch repair and the regulation of expression of some genes. It is intended to encompass dam with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "mutM" refers to an 8-oxoguanine DNA

30      glycosylase that removes 7,8-dihydro-8-oxoguanine (8-oxoG) and formamido pyrimidine (Fapy) lesions from DNA. It is intended to

encompass mutM with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "mutY" refers to an adenine glycosylase that is involved in the repair of 7,8-dihydro-8-oxo-2'-deoxyguanosine (OG):A and 5 G:A mispairs in DNA. It is intended to encompass mutY with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "TDG" refers to a thymine-DNA glycosylase that corrects G/T mispairs to G/C pairs. It is intended to encompass TDG with 10 conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "MUG" refers to a uracil-DNA glycosylase that corrects G/T and G/U mispairs to G/C pairs. It is intended to encompass MUG with conservative amino acid substitutions that do not substantially 15 alter its activity.

As used herein, "AlkA" refers to a 3-methyladenine DNA glycosylase II that corrects 5-formyluracil (fU)/G mispairs. It is intended to encompass AlkA with conservative amino acid substitutions that do not substantially alter its activity.

20 As used herein, "MSH2" refers to the common component of the eukaryotic DNA repair complex MSH2-MSH6 (MutS $\alpha$ ), which repairs base-base mispairs and insertion/deletion mispairs up to 12 unpaired bases, and the eukaryotic DNA repair complex MSH2-MSH3 (MutS $\beta$ ), which repairs insertion/deletion mispairs having two or more unpaired 25 bases but does not repair single base insertion/deletion mispairs. As used herein, "MSH3" refers to the unique component of the "MSH2-MSH3" complex and "MSH6" refers to the unique component of the "MSH2-MSH6" complex. It is intended to encompass MSH2, MSH3 and MSH 6 with conservative amino acid substitutions that do not substantially alter 30 its respective activity.

As used herein, "MLH1" and "PMS1" (PMS2 in humans) refers to

the components of the eukaryotic mutL-related protein complex, MLH1-PMS1, that interacts with MSH2-containing complexes bound to mispaired bases. It is intended to encompass MLH1 and PSM1 with conservative amino acid substitutions that do not substantially alter its  
5 respective activity.

As used herein, "exonuclease I" refers to an eukaryotic 5'→3' exonuclease that has a preference for degrading double-stranded DNA. Exonuclease I involves in the DNA repair via its interaction with MSH2. It is intended to encompass exonuclease I with conservative amino acid  
10 substitutions that do not substantially alter its respective activity.

As used herein, "T4 endonuclease V (EndoV)" refers to a base excision repair enzyme that removes thymine dimers (TD) from damaged DNA. It is intended to encompass T4 endonuclease V with conservative amino acid substitutions that do not substantially alter its respective  
15 activity.

As used herein, "FEN1 (rad27)" refers to an evolutionarily conserved component of DNA replication complex. FEN1 processes Okazaki fragments during replication and is involved in base excision repair. FEN1 removes the last primer ribonucleotide on the lagging strand  
20 and it cleaves a 5' flap that may result from strand displacement during replication or during base excision repair. It is intended to encompass FEN1 (rad27) with conservative amino acid substitutions that do not substantially alter its respective activity.

As used herein, "replication protein A (RPA)" refers to a  
25 heterotrimeric single-stranded DNA-binding protein that is highly conserved in eukaryotes. RPA plays essential roles in many aspects of nucleic acid metabolism, including DNA replication, nucleotide excision repair, and homologous recombination. It is intended to encompass RPA with conservative amino acid substitutions that do not substantially alter  
30 its respective activity.

As used herein, "proliferating cell nuclear antigen A (PCNA)" refers

to a DNA sliding clamp for DNA polymerase delta and is an essential component for eukaryotic chromosomal DNA replication. PCNA interacts with multiple partners, involved, for example, in Okazaki fragment joining, DNA repair, DNA methylation and chromatin assembly. PCNA is required

5 for nucleotide excision repair, base excision repair and mismatch repair. DNA polymerases, RFC and PCNA recognize 3' ends of gaped DNA and fill the gaps by the same mechanism as used for joining of Okazaki fragments. It is intended to encompass PCNA with conservative amino acid substitutions that do not substantially alter its respective activity.

10 As used herein, "replication factor C (RFC)" refers to a five-subunit protein complex required for coordinate leading and lagging strand DNA synthesis during S phase and DNA repair in eukaryotic cells. RFC functions to load the proliferating cell nuclear antigen (PCNA), a processivity factor for polymerases delta and epsilon, onto primed DNA

15 templates. This process, which is ATP-dependent, is carried out by 1) recognition of the primer terminus by RFC, 2) binding to and disruption of the PCNA trimer, and then 3) topologically linking the PCNA to the DNA. It is intended to encompass RFC with conservative amino acid substitutions that do not substantially alter its respective activity.

20 As used herein, "DNA polymerase  $\epsilon$ " refers to a mammalian DNA polymerase that has a tightly associated 3'-5' exonuclease activity. DNA polymerase  $\delta$  is required at least for the repair synthesis of UV-damaged DNA. It is intended to encompass DNA polymerase  $\epsilon$  with conservative amino acid substitutions that do not substantially alter its

25 respective activity.

As used herein, "DNA polymerase  $\delta$ " refers to a DNA polymerase that plays important roles in DNA replication, nucleotide excision repair, base excision repair and VDJ recombination. The function of DNA polymerase  $\delta$  must be considered in the context of two other factors,

30 PCNA and RFC, two protein complexes that build together the moving platform for DNA polymerase  $\delta$ . This moving platform provides an

important framework for dynamic properties of an accurate DNA polymerase  $\delta$ , such as its recruitment when its function is needed, the facilitation of DNA polymerase  $\delta$  binding to the primer terminus, the increase in DNA polymerase  $\delta$  processivity, the prevention of

5 non-productive binding of the DNA polymerase  $\delta$  to single-stranded DNA, the release of DNA polymerase  $\delta$  after DNA synthesis and the bridging of DNA polymerase  $\delta$  interactions to other replication proteins. It is intended to encompass DNA polymerase  $\delta$  with conservative amino acid substitutions that do not substantially alter its respective activity.

10 As used herein, "DNA polymerase III holoenzyme" refers to an enzyme that contains two DNA polymerases embedded in a particle with 9 other subunits. This multisubunit DNA polymerase is the *E. coli* chromosomal replicase, and it has several special features that distinguish it as a replicating machine. For example, one of its subunits is a circular protein that slides along DNA while clamping the rest of the machinery to the template. Other subunits act together as a matchmaker to assemble the ring onto DNA. Overall, *E. coli* DNA polymerase III holoenzyme is very similar in structure and function to the chromosomal replicases of eukaryotes, from yeast all the way up to humans..

15

20 As used herein, "mutation" refers to change(s) in the nucleic acid length and/or sequence in an organism, which may arise in any of a variety of different ways, e.g., frame-shift mutation, non-sense mutation or missense mutation.

As used herein, "disease or disorder" refers to a pathological

25 condition in an organism resulting from, e.g., infection or genetic defect, and characterized by identifiable symptoms.

As used herein, "cancer" refers to a pathological condition that occurs when cell division gets out of control. Usually, the timing of cell division is under strict constraint, involving a network of signals that

30 work together to say when a cell can divide, how often it should happen and how errors can be fixed. Mutations in one or more of the nodes in

this network can trigger cancer, be it through exposure to some environmental factor (e.g., tobacco smoke) or because of a genetic predisposition, or both. Usually, several cancer-promoting factors have to add up before a person will develop a malignant growth: with some

5 exceptions, no one risk alone is sufficient. The predominant mechanisms for the cancers are (i) impairment of a DNA repair pathway (ii) the transformation of a normal gene into an oncogene and (iii) the malfunction of a tumor suppressor gene.

As used herein, "an immune system disease or disorder" refers to a

10 pathological condition caused by a defect in the immune system. The immune system is a complex and highly developed system, yet its mission is simple: to seek and kill invaders. If a person is born with a severely defective immune system, death from infection by a virus, bacterium, fungus or parasite will occur. In severe combined

15 immunodeficiency, lack of an enzyme means that toxic waste builds up inside immune system cells, killing them and thus devastating the immune system. A lack of immune system cells is also the basis for DiGeorge syndrome: improper development of the thymus gland means that T cell production is diminished. Most other immune disorders result from either

20 an excessive immune response or an 'autoimmune attack'. For example, asthma, familial Mediterranean fever and Crohn disease (inflammatory bowel disease) all result from an over-reaction of the immune system, while autoimmune polyglandular syndrome and some facets of diabetes are due to the immune system attacking 'self' cells and molecules. A key

25 part of the immune system's role is to differentiate between invaders and the body's own cells - when it fails to make this distinction, a reaction against 'self' cells and molecules causes autoimmune disease.

As used herein, "a metabolism disease or disorder" refers to a pathological condition caused by errors in metabolic processes.

30 Metabolism is the means by which the body derives energy and synthesizes the other molecules it needs from the fats, carbohydrates and

proteins we eat as food, by enzymatic reactions helped by minerals and vitamins. There is a significant level of tolerance of errors in the system: often, a mutation in one enzyme does not mean that the individual will suffer from a disease. A number of different enzymes may compete to 5 modify the same molecule, and there may be more than one way to achieve the same end result for a variety of metabolic intermediates. Disease will only occur if a critical enzyme is disabled, or if a control mechanism for a metabolic pathway is affected.

As used herein, "a muscle and bone disease or disorder" refers to a 10 pathological condition caused by defects in genes important for the formation and function of muscles, and connective tissues. Connective tissue is used herein as a broad term that includes bones, cartilage and tendons. For example, defects in fibrillin - a connective tissue proteins that is important in making the tissue strong yet flexible - cause Marfan 15 syndrome, while diastrophic dysplasia is caused by a defect in a sulfate transporter found in cartilage. Two diseases that originate through a defect in the muscle cells themselves are Duchenne muscular dystrophy (DMD) and myotonic dystrophy (DM). DM is another 'dynamic mutation' disease, similar to Huntington disease, that involves the expansion of a 20 nucleotide repeat, this time in a muscle protein kinase gene. DMD involves a defect in the cytoskeletal protein, dystrophin, which is important for maintaining cell structure.

As used herein, "a nervous system disease or disorder" refers to a pathological condition caused by defects in the nervous system including 25 the central nervous system, *i.e.*, brain, and the peripheral nervous system. The brain and nervous system form an intricate network of electrical signals that are responsible for coordinating muscles, the senses, speech, memories, thought and emotion. Several diseases that directly affect the nervous system have a genetic component: some are 30 due to a mutation in a single gene, others are proving to have a more complex mode of inheritance. As our understanding of the pathogenesis

of neurodegenerative disorders deepens, common themes begin to emerge: Alzheimer brain plaques and the inclusion bodies found in Parkinson disease contain at least one common component, while Huntington disease, fragile X syndrome and spinocerebellar atrophy are all 5 'dynamic mutation' diseases in which there is an expansion of a DNA repeat sequence. Apoptosis is emerging as one of the molecular mechanisms invoked in several neurodegenerative diseases, as are other, specific, intracellular signaling events. The biosynthesis of myelin and the regulation of cholesterol traffic are also involved in Charcot-Marie-Tooth 10 and Neimann-Pick disease, respectively.

As used herein, "a signal disease or disorder" refers to a pathological condition caused by defects in the signal transduction process. Signal transduction within and between cells mean that they can communicate important information and act upon it. Hormones 15 released from their site of synthesis carry a message to their target site, as in the case of leptin, which is released from adipose tissue (fat cells) and transported via the blood to the brain. Here, the leptin signals that enough has been eaten. Leptin binds to a receptor on the surface of hypothalamus cells, triggering subsequent intracellular signaling networks. 20 Intracellular signaling defects account for several diseases, including cancers, ataxia telangiectasia and Cockayne syndrome. Faulty DNA repair mechanisms are also invoked in pathogenesis, since control of cell division, DNA synthesis and DNA repair all are inextricably linked. The end-result of many cell signals is to alter the expression of genes 25 (transcription) by acting on DNA-binding proteins. Some diseases are the result of a lack of or a mutation in these proteins, which stop them from binding DNA in the normal way. Since signaling networks impinge on so many aspects of normal function, it is not surprising that so many diseases have at least some basis in a signaling defect.

30 As used herein, "a transporter disease or disorder" refers to a pathological condition caused by defects in a transporter, channel or

pump. Transporters, channels or pumps that reside in cell membranes are key to maintaining the right balance of ions in cells, and are vital for transmitting signals from nerves to tissues. The consequences of defects in ion channels and transporters are diverse, depending on where they are located and what their cargo is. For example, in the heart, defects in potassium channels do not allow proper transmission of electrical impulses, resulting in the arrhythmia seen in long QT syndrome. In the lungs, failure of a sodium and chloride transporter found in epithelial cells leads to the congestion of cystic fibrosis, while one of the most common inherited forms of deafness, Pendred syndrome, looks to be associated with a defect in a sulphate transporter.

As used herein, "virus" refers to obligate intracellular parasites of living but non-cellular nature, that contain DNA or RNA and a protein coat. Viruses range in diameter from about 20 to about 300 nm. Class I viruses (Baltimore classification) have a double-stranded DNA as their genome; Class II viruses have a single-stranded DNA as their genome; Class III viruses have a double-stranded RNA as their genome; Class IV viruses have a positive single-stranded RNA as their genome, the genome itself acting as mRNA; Class V viruses have a negative single-stranded RNA as their genome used as a template for mRNA synthesis; and Class VI viruses have a positive single-stranded RNA genome but with a DNA intermediate not only in replication but also in mRNA synthesis. The majority of viruses are recognized by the diseases they cause in plants, animals and prokaryotes. Viruses of prokaryotes are known as bacteriophages.

As used herein, "bacteria" refers to small prokaryotic organisms (linear dimensions of around 1  $\mu\text{m}$ ) with non-compartmentalized circular DNA and ribosomes of about 70S. Bacteria protein synthesis differs from that of eukaryotes. Many anti-bacterial antibiotics interfere with bacteria proteins synthesis but do not affect the infected host.

As used herein, "eubacteria" refers to a major subdivision of the

bacteria except the archaebacteria. Most Gram-positive bacteria, cyanobacteria, mycoplasmas, enterobacteria, pseudomonas and chloroplasts are eubacteria. The cytoplasmic membrane of eubacteria contains ester-linked lipids; there is peptidoglycan in the cell wall (if present); and no introns have been discovered in eubacteria.

As used herein, "archaebacteria" refers to a major subdivision of the bacteria except the eubacteria. There are 3 main orders of archaebacteria: extreme halophiles, methanogens and sulphur-dependent extreme thermophiles. Archaeabacteria differs from eubacteria in 10 ribosomal structure, the possession (in some case) of introns, and other features including membrane composition.

As used herein, "locus" refers to the site in linkage map or on a chromosome where the nucleic acid sequence, e.g., gene, for a particular trait is located. Any one of the alleles of a sequence may be present at 15 this site.

As used herein, "an allele" refers to one of any different forms or variants of a gene found at the same place, or a locus, on a chromosome.

As used herein, "polymorphism" refers to the existence, in a 20 population, of two or more alleles of a nucleic acid sequence, e.g., gene, where the frequency of the rarer alleles is greater than can be explained by recurrent mutation alone (typically greater than 1%).

As used herein, "variable nucleotide type polymorphism ("VNTR")" refers to polymorphisms arising from spontaneous tandem duplications 25 of di- or trinucleotide repeated motifs of nucleotides.

As used herein, "single nucleotide polymorphism ("SNP")" refers to polymorphisms arising from the replacement of only a single nucleotide from the initially present gene sequence.

As used herein, "enzymatic amplification" refers to an enzyme-30 catalyzed reaction by which nucleic acid, e.g., DNA, molecules are amplified. Examples of such reactions include the polymerase chain

reaction and reactions utilizing reverse transcription and subsequent DNA amplification of one or more expressed RNA sequences.

As used herein, "exonuclease" refers to an enzyme that cleaves nucleotides one at time from the end of a polynucleotide chain.

5 Exonuclease may be specific for either 5' or 3' end of DNA or RNA. If protein is bound to the nucleic acid, exonuclease cleavage stops when the exonuclease encounters the protein.

As used herein, "recombinase" refers to an enzyme that catalyzes the inter-molecular formation of a nucleic acid duplex from

10 single-stranded nucleic acids obtained from different sources, by a renaturation reaction. Such a recombinase is also capable of catalyzing a strand transfer reaction between a single-stranded nucleic acid from one source and double-stranded nucleic acid obtained from a different source.

As used herein, "serum" refers to the fluid portion of the blood  
15 obtained after removal of the fibrin clot and blood cells, distinguished from the plasma in circulating blood.

As used herein, "plasma" refers to the fluid, noncellular portion of the blood, distinguished from the serum obtained after coagulation.

As used herein, "substantially pure" means sufficiently  
20 homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not  
25 detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound may, however, be a mixture of stereoisomers or isomers. In  
30 such instances, further purification might increase the specific activity of the compound.

As used herein, "biological activity" refers to the in vivo activities of a compound or physiological responses that result upon in vivo administration of a compound, composition or other mixture. Biological activity, thus, encompasses therapeutic effects and pharmaceutical

5 activity of such compounds, compositions and mixtures. Biological activities may be observed in vitro systems designed to test or use such activities. Thus, for purposes herein the biological activity of a luciferase is its oxygenase activity whereby, upon oxidation of a substrate, light is produced.

10 As used herein, a "receptor" refers to a molecule that has an affinity for a given ligand. Receptors may be naturally-occurring or synthetic molecules. Receptors may also be referred to in the art as anti-ligands. As used herein, the receptor and anti-ligand are interchangeable. Receptors can be used in their unaltered state or as aggregates with other

15 species. Receptors may be attached, covalently or noncovalently, or in physical contact with, to a binding member, either directly or indirectly via a specific binding substance or linker. Examples of receptors, include, but are not limited to: antibodies, cell membrane receptors surface receptors and internalizing receptors, monoclonal antibodies and antisera

20 reactive with specific antigenic determinants [such as on viruses, cells, or other materials], drugs, polynucleotides, nucleic acids, peptides, cofactors, lectins, sugars, polysaccharides, cells, cellular membranes, and organelles.

Examples of receptors and applications using such receptors,

25 include but are not restricted to:

a) enzymes: specific transport proteins or enzymes essential to survival of microorganisms, which could serve as targets for antibiotic [ligand] selection;

b) antibodies: identification of a ligand-binding site on the antibody

30 molecule that combines with the epitope of an antigen of interest may be investigated; determination of a sequence that mimics an antigenic

epitope may lead to the development of vaccines of which the immunogen is based on one or more of such sequences or lead to the development of related diagnostic agents or compounds useful in therapeutic treatments such as for auto-immune diseases

- 5       c) nucleic acids: identification of ligand, such as protein or RNA, binding sites;
- d) catalytic polypeptides: polymers, preferably polypeptides, that are capable of promoting a chemical reaction involving the conversion of one or more reactants to one or more products; such polypeptides
- 10      generally include a binding site specific for at least one reactant or reaction intermediate and an active functionality proximate to the binding site, in which the functionality is capable of chemically modifying the bound reactant [see, e.g., U.S. Patent No. 5,215,899];
- 15      e) hormone receptors: determination of the ligands that bind with high affinity to a receptor is useful in the development of hormone replacement therapies; for example, identification of ligands that bind to such receptors may lead to the development of drugs to control blood pressure; and
- 20      f) opiate receptors: determination of ligands that bind to the opiate receptors in the brain is useful in the development of less-addictive replacements for morphine and related drugs.

As used herein, "antibody" includes antibody fragments, such as Fab fragments, which are composed of a light chain and the variable region of a heavy chain.

- 25      As used herein, "humanized antibodies" refer to antibodies that are modified to include "human" sequences of amino acids so that administration to a human will not provoke an immune response. Methods for preparation of such antibodies are known. For example, the hybridoma that expresses the monoclonal antibody is altered by
- 30      recombinant DNA techniques to express an antibody in which the amino acid composition of the non-variable regions is based on human

antibodies. Computer programs have been designed to identify such regions.

As used herein, "production by recombinant means" refers to production methods that use recombinant nucleic acid methods that rely 5 on well known methods of molecular biology for expressing proteins encoded by cloned nucleic acids.

As used herein, "substantially identical" to a product means sufficiently similar so that the property of interest is sufficiently unchanged so that the substantially identical product can be used in place 10 of the product.

As used herein, "equivalent," when referring to two sequences of nucleic acids means that the two sequences in question encode the same sequence of amino acids or equivalent proteins. It also encompasses those that hybridize under conditions of moderate, preferably high 15 stringency, whereby the encoded protein retains desired properties.

As used herein, when "equivalent" is used in referring to two proteins or peptides, it means that the two proteins or peptides have substantially the same amino acid sequence with only conservative amino acid substitutions (see, e.g., Table 1, above) that do not substantially 20 alter the activity or function of the protein or peptide.

When "equivalent" refers to a property, the property does not need to be present to the same extent [e.g., two peptides can exhibit different rates of the same type of enzymatic activity], but the activities are preferably substantially the same. "Complementary," when referring 25 to two nucleic acid molecules, means that the two sequences of nucleotides are capable of hybridizing, preferably with less than 25%, more preferably with less than 15%, even more preferably with less than 5%, most preferably with no mismatches between opposed nucleotides. Preferably the two molecules will hybridize under conditions of high 30 stringency.

As used herein: "stringency of hybridization" in determining percentage mismatch is as follows:

- 1) high stringency: 0.1 x SSPE, 0.1% SDS, 65°C;
- 2) medium stringency: 0.2 x SSPE, 0.1% SDS, 50°C (also
- 5 referred to as moderate stringency); and
- 3) low stringency: 1.0 x SSPE, 0.1% SDS, 50°C.

It is understood that equivalent stringencies may be achieved using alternative buffers, salts and temperatures.

The term "substantially" identical or homologous or similar varies  
10 with the context as understood by those skilled in the relevant art and generally means at least 70%, preferably means at least 80%, more preferably at least 90%, and most preferably at least 95% identity.

As used herein, a "composition" refers to any mixture of two or more products or compounds. It may be a solution, a suspension, liquid,  
15 powder, a paste, aqueous, non-aqueous or any combination thereof.

As used herein, a "combination" refers to any association between two or among more items.

As used herein, "fluid" refers to any composition that can flow. Fluids thus encompass compositions that are in the form of semi-solids,  
20 pastes, solutions, aqueous mixtures, gels, lotions, creams and other such compositions.

As used herein, "vector (or plasmid)" refers to discrete elements that are used to introduce heterologous DNA into cells for either expression or replication thereof. Selection and use of such vehicles are  
25 well known within the skill of the artisan. An expression vector includes vectors capable of expressing DNAs that are operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a  
30 phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA.

Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

5 As used herein, "a promoter region or promoter element" refers to a segment of DNA or RNA that controls transcription of the DNA or RNA to which it is operatively linked. The promoter region includes specific sequences that are sufficient for RNA polymerase recognition, binding and transcription initiation. This portion of the promoter region is referred  
10 to as the promoter. In addition, the promoter region includes sequences that modulate this recognition, binding and transcription initiation activity of RNA polymerase. These sequences may be *cis* acting or may be responsive to *trans* acting factors. Promoters, depending upon the nature of the regulation, may be constitutive or regulated. Exemplary promoters  
15 contemplated for use in prokaryotes include the bacteriophage T7 and T3 promoters, and the like.

As used herein, "operatively linked or operationally associated" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional  
20 and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the  
25 DNA. In order to optimize expression and/or *in vitro* transcription, it may be necessary to remove, add or alter 5' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (*i.e.*, start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation.  
30 Alternatively, consensus ribosome binding sites (see, *e.g.*, Kozak, *J. Biol. Chem.*, 266:19867-19870 (1991)) can be inserted immediately 5' of the

start codon and may enhance expression. The desirability of (or need for) such modification may be empirically determined.

As used herein, "sample" refers to anything which may contain an analyte for which an analyte assay is desired. The sample may be a

5 biological sample, such as a biological fluid or a biological tissue.

Examples of biological fluids include urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, mucus, amniotic fluid or the like. Biological tissues are aggregates of cells, usually of a particular kind together with their intercellular substance that form one of

10 the structural materials of a human, animal, plant, bacterial, fungal or viral structure, including connective, epithelium, muscle and nerve tissues.

Examples of biological tissues also include organs, tumors, lymph nodes, arteries and individual cell(s).

As used herein, "replication" refers to a process of DNA-dependent

15 DNA synthesis wherein the DNA molecule is duplicated to give identical copies.

As used herein, "transcription" refers to a process of DNA-dependent RNA synthesis.

As used herein, "recombination" refers to a reaction between

20 homologous sequences of DNA. The critical feature is that the enzymes responsible for recombination can use any pair of homologous sequences as substrates, although some types of sequences may be favored over others. Recombination allows favorable or unfavorable mutations to be separated and tested as individual units in new assortments.

25 As used herein, "DNA structure maintenance" refers to DNA sequences, through binding to proteins, that maintain the DNA molecule in particular structures such as chromatids, chromatin or chromosomes.

As used herein, "DNA polymerase" refers to an enzyme that synthesizes DNA using a DNA as the template. It is intended to

30 encompass DNA polymerase with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "DNA-dependent RNA polymerase" or "transcriptase" refers to an enzyme that synthesizes RNA using a DNA as the template. It is intended to encompass DNA-dependent RNA polymerase with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "DNAase" refers to an enzyme that attacks bonds in DNA. It is intended to encompass DNAase with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "DNA ligase" refers to an enzyme that catalyses the formation of a phosphodiester bond to link two adjacent bases separated by a nick in one strand of double helix of DNA. It is intended to encompass DNA ligase with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "DNA topoisomerase" refers to an enzyme that can change the linking number of DNA. It is intended to encompass DNA topoisomerase with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "DNA transposase" refers to an enzyme that is involved in insertion of a transposon at a new site. It is intended to encompass DNA transposase with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "Transposon" refers to a DNA sequence that is able to replicate and insert one copy at a new location in the genome.

As used herein, "DNA kinase" refers to an enzyme that phosphorylates DNA. It is intended to encompass DNA kinase with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "restriction enzyme" refers to an enzyme that recognizes specific short sequences of DNA and cleaves the duplex at the recognition site or other site. It is intended to encompass a restriction enzyme with conservative amino acid substitutions that do not

substantially alter its activity.

As used herein, "rRNA" or "ribosomal RNA" refers to the RNA components of the ribosome, a compact ribonucleoprotein particle that assembles amino acids into proteins.

5 As used herein, "mRNA" or "messenger RNA" refers to the RNA molecule that bears the same sequence of the DNA coding strand and is used as the template in protein synthesis.

As used herein, "tRNA" or "transfer RNA" refers to the RNA molecule that carries amino acids to the ribosome for protein synthesis.

10 As used herein, "reverse transcription" refers to the RNA-dependent DNA synthesis.

As used herein, "RNA splicing" refers to the removal of introns and joining of exons in RNA so that introns are spliced out and exons are spliced together.

15 As used herein, "RNA-dependent DNA polymerase" or "reverse transcriptase" refers to an enzyme that synthesizes DNA using a RNA as the template. It is intended to encompass a RNA-dependent DNA polymerase with conservative amino acid substitutions that do not substantially alter its activity.

20 As used herein, "RNA-dependent RNA polymerase" refers to an enzyme that synthesizes RNA using a RNA as the template. It is intended to encompass a RNA-dependent RNA polymerase with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "RNA ligase" refers to an enzyme that catalyses 25 the formation of a phosphodiester bond to link two adjacent bases separated by a nick in one strand of RNA. It is intended to encompass a RNA ligase with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "RNA maturase" refers to an enzyme that 30 catalyses the removal of intron in the RNA splicing. It is intended to encompass a RNA maturase with conservative amino acid substitutions

that do not substantially alter its activity.

As used herein, "luminescence" refers to the detectable EM radiation, generally, UV, IR or visible EM radiation that is produced when the excited product of an exergic chemical process reverts to its ground state with the emission of light. Chemiluminescence is luminescence that results from a chemical reaction. Bioluminescence is chemiluminescence that results from a chemical reaction using biological molecules or synthetic versions or analogs thereof as substrates and/or enzymes.

As used herein, "bioluminescence," which is a type of chemiluminescence, refers to the emission of light by biological molecules, particularly proteins. The essential condition for bioluminescence is molecular oxygen, either bound or free in the presence of an oxygenase, a luciferase, which acts on a substrate, a luciferin. Bioluminescence is generated by an enzyme or other protein (luciferase) that is an oxygenase that acts on a substrate luciferin (a bioluminescence substrate) in the presence of molecular oxygen and transforms the substrate to an excited state, which upon return to a lower energy level releases the energy in the form of light.

As used herein, the substrates and enzymes for producing bioluminescence are generically referred to as luciferin and luciferase, respectively. When reference is made to a particular species thereof, for clarity, each generic term is used with the name of the organism from which it derives, for example, bacterial luciferin or firefly luciferase.

As used herein, "luciferase" refers to oxygenases that catalyze a light emitting reaction. For instance, bacterial luciferases catalyze the oxidation of flavin mononucleotide [FMN] and aliphatic aldehydes, which reaction produces light. Another class of luciferases, found among marine arthropods, catalyzes the oxidation of *Cypridina* [*Vargula*] luciferin, and another class of luciferases catalyzes the oxidation of *Coleoptera* luciferin.

Thus, luciferase refers to an enzyme or photoprotein that catalyzes a bioluminescent reaction [a reaction that produces bioluminescence]. The luciferases, such as firefly and *Renilla* luciferases, that are enzymes which act catalytically and are unchanged during the bioluminescence generating reaction. The luciferase photoproteins, such as the aequorin photoprotein to which luciferin is non-covalently bound, are changed, such as by release of the luciferin, during bioluminescence generating reaction. The luciferase is a protein that occurs naturally in an organism or a variant or mutant thereof, such as a variant produced by mutagenesis that has one or more properties, such as thermal stability, that differ from the naturally-occurring protein. Luciferases and modified mutant or variant forms thereof are well known. For purposes herein, reference to luciferase refers to either the photoproteins or luciferases.

As used herein, "peroxidase" refers to an enzyme that catalyses a host of reactions in which hydrogen peroxide is a specific oxidizing agent and a wide range of substrates act as electron donors. It is intended to encompass a peroxidase with conservative amino acid substitutions that do not substantially alter its activity. Peroxidases are widely distributed in nature and are produced by a wide variety of plant species. The chief commercially available peroxidase is horseradish peroxidase.

As used herein, "urease" refers to an enzyme that catalyses decomposition of urea to form ammonia and carbon dioxide. It is intended to encompass an urease with conservative amino acid substitutions that do not substantially alter its activity. Urease is widely found in plants, animals and microorganisms.

As used herein, "alkaline phosphatases" refers to a family of functionally related enzymes named after the tissues in which they predominately appear. Alkaline phosphatases carry out hydrolase/transferase reactions on phosphate-containing substrates at a high pH optimum. It is intended to encompass an alkaline phosphatases with conservative amino acid substitutions that do not substantially alter

its activity.

As used herein, "glutathione S-transferase" refers to a ubiquitous family of enzymes with dual substrate specificities that perform important biochemical functions of xenobiotic biotransformation and detoxification, 5 drug metabolism, and protection of tissues against peroxidative damage. The basic reaction catalyzed by glutathione S-transferase is the conjugation of an electrophile with reduced glutathione (GSH) and results in either activation or deactivation/detoxification of the chemical. It is intended to encompass a glutathione S-transferase with conservative 10 amino acid substitutions that do not substantially alter its activity.

As used herein, high-throughput screening (HTS) refers to processes that test a large number of samples, such as samples of diverse chemical structures against disease targets to identify "hits" (see, e.g., Broach *et al.* High throughput screening for drug discovery, *Nature*, 15 384:14-16 (1996); Janzen, *et al.* High throughput screening as a discovery tool in the pharmaceutical industry, *Lab Robotics Automation*: 8261-265 (1996); Fernandes, P.B., Letter from the society president, *J. Biomol. Screening*, 2:1 (1997); Burbaum, *et al.*, New technologies for high-throughput screening, *Curr. Opin. Chem. Biol.*, 1:72-78 (1997)). 20 HTS operations are highly automated and computerized to handle sample preparation, assay procedures and the subsequent processing of large volumes of data.

As used herein, the abbreviations for any protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord 25 with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, (1972) Biochem., 11:1726).

For clarity of disclosure, and not by way of limitation, the detailed description is divided into the subsections that follow.

**B. METHODS FOR DETECTING ABNORMAL BASE-PAIRING**

Provided herein are methods for detecting abnormal base-pairing in a nucleic acid duplex. Detection of abnormal base pairing has numerous applications, such as in diagnostics, mutational analyses and

5 polymorphism identification. The method involves binding a mutant enzyme that specifically binds to mismatched base pairs in a DNA duplex, DNA:RNA duplex, or RNA:RNA duplex, and detecting such binding, which can be quantitative. By virtue of the base specificity of the certain enzymes the identity of the abnormal base pairing may be determined.

10 The reactions can be performed in various formats, including solution and solid phase reactions. Solid supports to which nucleic acid or enzyme is bound. In addition, the resulting complexes of enzyme bound to nucleic acid can be captured on solid supports by virtue of interaction of the nucleic acid with other nucleic acids on the supports or

15 the enzyme with moieties on the supports.

The preferred formats herein are those that are amenable to high throughput analyses, such as chip-based reactions in which nucleic acid probes of known sequence are arranged, such as in an array on a support, and reacted with a sample, such as nucleic acid from a body

20 fluid or tissue.

In a particular embodiment, the method is performed by contacting a nucleic acid duplex having or suspected of having an abnormal base-pairing with a mutant DNA repair enzyme or complex thereof, where the mutant DNA repair enzyme or complex thereof has 25 binding affinity for the abnormal base-pairing in the duplex but has attenuated catalytic activity; and then detecting binding between the nucleic acid duplex and the mutant DNA repair enzyme or complex thereof, whereby the presence or quantity of the abnormal base-pairing in the duplex is assessed.

30 As noted, the nucleic acid duplex to be assayed is a DNA:DNA, a DNA:RNA or a RNA:RNA duplex. Preferably, the nucleic acid duplex to

be assayed is a DNA:DNA duplex. The abnormal base-pairing to be detected includes a base-pair mismatch, a base insertion, a base deletion and a pyrimidine dimer. Preferably, the base-pair mismatch to be detected is a single base-pair mismatch. Non-limiting examples of the 5 base-pair mismatch that can be detected include A:A, A:C, A:G, C:C, C:T, G:G, G:T, T:T, C:U, G:U, T:U, U:U, 5-formyluracil (fU):G, 7,8-dihydro-8-oxo-guanine (8-oxoG):C, 8-oxoG:A or a combination thereof. Also preferably, the base insertion or base deletion to be detected is a single base insertion or deletion. For example, the base 10 insertion or base deletion resulting in a single-stranded loop containing about 1-5 bases or a loop containing more than 5 bases can be detected.

#### 1. MUTANT DNA REPAIR ENZYME OR COMPLEX THEREOF

Any mutant DNA repair enzyme or complex thereof that has binding affinity for the abnormal base-pairing in the duplex but has 15 attenuated catalytic activity can be used in the present methods. Such enzymes may be prepared by mutagenesis of nucleic acids encoding the enzyme and selection of the expressed protein for the requisite binding properties and reduced or absent catalytic activities.

Mutant enzymes having the desired specificity can be prepared 20 using routine mutagenesis methods. Residues to mutate can be identified by systematically mutating residues to different residues, and identifying those that have the desired reduction in catalytic activity and retention of binding activity for a particular abnormal base-pairing. Alternatively or additionally, mutations may be based upon predicted or known 3-D 25 structures of enzymes, including predicted affects of various mutations (see, e.g., Turner *et al.* (1998) Nature Structural Biol. **5**:369-376; Ault-Richié *et al.* (1994) J. Biol. Chem. **269**:31472-31478; Yuan *et al.* (1996) J. Biol. Chem. **271**:28009-28016; Williams *et al.* (1998) Biochemistry **37**:7096; Steadman *et al.* (1998) Biochemistry **37**:7089-30 7095; Finer-Moore *et al.* (1998) J. Mol. Biol. **276**:113-129; Strop *et al.* (1997) Protein Sci. **6**:2504-2511; Finer-Moore *et al.* (1996) Biochemistry

35:5125-5136; Schiffer *et al.* (1995) *Biochemistry* 34:16279-16287; Costi *et al.* (1996) *Biochemistry* 35:3944-3949; Graves *et al.* (1992) *Biochemistry* 31:15-21; Carreras *et al.* (1992) *Biochemistry* 31:6038-6044). Such predictions can be made by those of skill in the art of computational chemistry. Hence, for any selected enzyme, the mutations need to inactivate catalytic activity but retain binding activity can be determined empirically.

Mutant enzymes can be selected for example by plating plasmids containing DNA containing mutagenized genes in wells coated with duplexes containing mismatches, expressing the proteins, and looking for binding to the mismatched duplexes, and selecting the nucleic acid that expressed the proteins that bound thereto.

A typical mutant enzyme, is a DNA repair enzyme with a mutation that attenuates the catalytic activity, but that has little or small effects on the binding activity. By selecting the enzymes that bind to duplexes, which are retained on a support, enzymes with the desired specificity and lack of catalytic activity will be selected. Enzymes that retain catalytic activity, will not remain bound.

Exemplary DNA repair enzyme and complexes thereof that can be mutated for use in the methods herein, include, but are not limited to, a mutant mutH, a mutant mutL, a mutant mutM, a mutant mutS, a mutant mutY, a mutant uvrD, a mutant dam, a mutant thymidine DNA glycosylase (TDG), a mutant mismatch-specific DNA glycosylase (MUG), a mutant AlkA, a mutant MLH1, a mutant MSH2, a mutant MSH3, a mutant MSH6, a mutant Exonuclease I, a mutant T4 endonuclease V, a mutant FEN1 (RAD27), a mutant DNA polymerase  $\delta$ , a mutant DNA polymerase  $\epsilon$ , a mutant RPA, a mutant PCNA, a mutant RFC, a mutant Exonuclease V, a mutant DNA polymerase III holoenzyme, a mutant DNA helicase, a mutant RecJ exonuclease or a combination thereof.

a. Nucleic acids encoding DNA repair enzymes

Nucleic acids encoding DNA repair enzymes can be obtained by methods known in the art. Known nucleic acid sequences of DNA repair enzymes can be used in isolating nucleic acids encoding DNA repair 5 enzymes from natural or other sources. Alternatively, complete or partial nucleic acids encoding DNA repair enzymes can be obtained by chemical synthesis according to the known sequences or obtained from commercial or other sources.

Eukaryotic cells and prokaryotic cells can serve as a nucleic acid 10 source for the isolation of nucleic acids encoding DNA repair enzymes. The DNA can be obtained by standard procedures known in the art from cloned DNA (e.g., a DNA "library"), chemical synthesis, cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell (see, for example, Sambrook et al., 1989, Molecular Cloning, 15 A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II.). Clones derived from genomic DNA can contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA or RNA contain 20 only exon sequences. Whatever the source, the gene is generally molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from cDNA, cDNA can be generated from total cellular RNA or mRNA by methods that are known in the art. The gene can also be obtained from genomic DNA, where DNA 25 fragments are generated (e.g., using restriction enzymes or by mechanical shearing), some of which will encode the desired gene. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

30 Once the DNA fragments are generated, identification of the specific DNA fragment containing all or a portion of the DNA repair

enzymes gene can be accomplished in a number of ways.

A preferred method for isolating an DNA repair enzyme gene is by the polymerase chain reaction (PCR), which can be used to amplify the desired DNA repair enzyme sequence in a genomic or cDNA library or 5 from genomic DNA or cDNA that has not been incorporated into a library. Oligonucleotide primers which hybridize to the DNA repair enzyme sequences can be used as primers in PCR.

Additionally, a portion of the DNA repair enzyme (of any species) gene or its specific RNA, or a fragment thereof, can be purified (or an 10 oligonucleotide synthesized) and labeled, the generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton, W. and Davis, R., 1977, *Science* 196:180; Grunstein, M. And Hogness, D., 1975, *Proc. Natl. Acad. Sci. U.S.A.* 72:3961). Those DNA 15 fragments with substantial homology to the probe will hybridize. The DNA repair enzyme nucleic acids can be also identified and isolated by expression cloning using, for example, DNA repair activities or anti-DNA repair enzyme antibodies for selection.

Alternatives to obtaining the DNA repair enzyme DNA by cloning or amplification include, but are not limited to, chemically synthesizing the 20 gene sequence itself from the known DNA repair enzyme nucleotide sequence or making cDNA to the mRNA which encodes the DNA repair enzyme. Any suitable method known to those of skill in the art may be employed.

Once a clone has been obtained, its identity can be confirmed by 25 nucleic acid sequencing (by methods known in the art) and comparison to known DNA repair enzyme sequences. DNA sequence analysis can be performed by techniques known in the art, including but not limited to, the method of Maxam and Gilbert (1980, *Meth. Enzymol.* 65:499-560), the Sanger dideoxy method (Sanger, F., et al., 1977, *Proc. Natl. Acad. 30 Sci. U.S.A.* 74:5463), the use of T7 DNA polymerase (Tabor and Richardson, U.S. Patent No. 4,795,699), use of an automated DNA

sequenator (e.g., Applied Biosystems, Foster City, CA).

Nucleic acids which are hybridizable to a DNA repair enzyme nucleic acid, or to a nucleic acid encoding an DNA repair enzyme derivative can be isolated, by nucleic acid hybridization under conditions 5 of low, high, or medium stringency (Shilo and Weinberg, 1981, *Proc. Natl. Acad. Sci. USA* 78:6789-6792).

b. Selecting and producing mutant DNA repair enzymes

Once nucleic acids encoding the DNA repair enzymes are obtained, these nucleic acids can be mutagenized and screened and/or selected for 10 DNA repair enzymes that substantially retain their binding affinity or have enhanced binding affinity for abnormal base-pairing but have attenuated catalytic activity. Insertion, deletion or point mutation(s) can be introduced into nucleic acids encoding the DNA repair enzymes. Techniques for mutagenesis known in the art can be used, including, but 15 not limited to, *in vitro* site-directed mutagenesis (Hutchinson et al., 1978, *J. Biol. Chem.* 253:6551), use of TAB<sup>®</sup> linkers (Pharmacia), mutation-containing PCR primers, etc. Mutagenesis can be followed by phenotypic testing of the altered gene product.

Site-directed mutagenesis protocols can take advantage of vectors 20 that provide single stranded as well as double stranded DNA, as needed. Generally, the mutagenesis protocol with such vectors is as follows. A mutagenic primer, i.e., a primer complementary to the sequence to be changed, but including one or a small number of altered, added, or deleted bases, is synthesized. The primer is extended *in vitro* by a DNA 25 polymerase and, after some additional manipulations, the now double-stranded DNA is transfected into bacterial cells. Next, by a variety of methods, the desired mutated DNA is identified, and the desired protein is purified from clones containing the mutated sequence. For longer sequences, additional cloning steps are often required because long 30 inserts (longer than 2 kilobases) are unstable in those vectors. Protocols are known to one skilled in the art and kits for site-directed mutagenesis

are widely available from biotechnology supply companies, for example from Amersham Life Science, Inc. (Arlington Heights, IL) and Stratagene Cloning Systems (La Jolla, CA).

Information regarding to the structural-function relationship of the

- 5 DNA repair enzymes can be used in the mutagenesis and selection of DNA repair enzymes that substantially retain their binding affinity or have enhanced binding affinity for the abnormal base-pairing but have attenuated catalytic activity. For example, mutants can be made in the enzyme's binding site for its co-enzyme, co-factor, or in the mutant
- 10 enzyme's catalytic site, or a combination thereof.

Once a mutant DNA repair enzyme with desired properties, *i.e.*, substantially retaining its binding affinity or having enhanced binding affinity for the abnormal base-pairing but has attenuated catalytic activity, is identified, such mutant DNA repair enzyme can be produced

- 15 by any methods known in the art including recombinant expression, chemical synthesis or a combination thereof. Preferably, the mutant DNA repair enzyme is obtained by recombinant expression.

For recombinant expression, the mutant DNA repair enzyme gene or portion thereof is inserted into an appropriate cloning vector for

- 20 expression in a particular host cell. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cells used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such
- 25 as pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene). The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. If, however, the complementary restriction sites used to fragment the DNA are not present in the cloning
- 30 vector, the ends of the DNA molecules can be enzymatically modified. Alternatively, a desired site can be produced by ligating sequences of

nucleotides (linkers) onto the DNA termini; these ligated linkers can include specific oligonucleotides encoding restriction endonuclease recognition sequences. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc.,  
5 so that many copies of the gene sequence are generated.

In an alternative method, the desired gene can be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the desired gene, for example, by size fractionation, can be done before insertion into the cloning vector.

10 In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated mutant DNA repair enzyme gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene can be obtained in large quantities by growing transformants, isolating the  
15 recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The nucleotide sequence coding for a mutant DNA repair enzyme or a functionally active analog or fragment or other derivative thereof, can  
20 be inserted into an appropriate expression vector, e.g., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native mutant DNA repair enzyme gene and/or its flanking regions. A variety of host-vector  
25 systems can be utilized to express the protein-coding sequence. These systems include but are not limited to mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage,  
30 DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector

system utilized, suitable transcription and translation elements can be used.

The methods previously described for the insertion of DNA fragments into a vector can be used to construct expression vectors containing a chimeric gene containing appropriate transcriptional/translational control signals and the protein coding sequences. These methods can include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of a nucleic acid sequence encoding a mutant DNA repair enzyme or peptide fragment can be regulated by a second nucleic acid sequence so that the mutant DNA repair enzyme or peptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a mutant DNA repair enzyme can be controlled by a promoter/enhancer element as is known in the art. Promoters which can be used to control a mutant DNA repair enzyme expression include, but are not limited to, the SV40 early promoter region (Bernouist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445), the regulatory sequences of the metallothioneine gene (Brinster et al., 1982, *Nature* 296:39-42); prokaryotic expression vectors such as the  $\beta$ -lactamase promoter (Villa-Kamaroff, et al., 1978, *Proc. Natl. Acad. Sci. U.S.A.* 75:3727-3731), or the *tac* promoter (DeBoer, et al., 1983, *Proc. Natl. Acad. Sci. U.S.A.* 80:21-25); see also "Useful proteins from recombinant bacteria" in *Scientific American*, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and certain animal transcriptional control regions.

For example, a vector can be used that contains a promoter

operably linked to a nucleic acid encoding a mutant DNA repair enzyme, one or more origins of replication, and, optionally, one or more selectable markers (e.g., an antibiotic resistance gene).

In a specific embodiment, an expression construct is made by

5 subcloning a mutant DNA repair enzyme coding sequence into the *Eco*RI restriction site of each of the three pGEX vectors (Glutathione S-Transferase expression vectors; see, e.g., Smith and Johnson, 1988, *Gene* 7:31-40). This allows for the expression of a mutant DNA repair enzyme product from the subclone in the correct reading frame.

10 Expression vectors containing a mutant DNA repair enzyme gene inserts can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a mutant DNA repair enzyme gene inserted in an expression vector can be

15 detected by nucleic acid hybridization using probes containing sequences that are homologous to an inserted mutant DNA repair enzyme gene. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to

20 antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of a mutant DNA repair enzyme gene in the vector. For example, if the mutant DNA repair enzyme gene is inserted within the marker gene sequence of the vector, recombinants containing the mutant DNA repair enzyme insert can be identified by the

25 absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the mutant DNA repair enzyme product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the mutant DNA repair enzyme in *in vitro* assay systems, e.g., binding with

30 anti-mutant DNA repair enzyme antibody.

Once a particular recombinant DNA molecule is identified and

isolated, several methods known in the art can be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be 5 used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (e.g., lambda), and plasmid and cosmid DNA vectors, to name but a few.

In addition, a host cell strain can be chosen which modulates the 10 expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered mutant DNA repair enzyme can be controlled. Furthermore, different host cells have characteristic and 15 specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, phosphorylation) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an 20 unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in appropriate animal cells can be used to ensure "native" glycosylation of a heterologous protein. Furthermore, different vector/host expression systems can effect processing reactions to different extent.

25

c. Mutant mutL or MLH1

In a specific embodiment, a mutant mutL or MLH1 is used in the present methods. The nucleic acid molecules containing sequences of 30 nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding mutL and in mutagenesis: AF170912

(*Caulobacter crescentus*), AI518690 (*Drosophila melanogaster*), AI456947 (*Drosophila melanogaster*), AI389544 (*Drosophila melanogaster*), AI387992 (*Drosophila melanogaster*), AI292490 (*Drosophila melanogaster*), AF068271 (*Drosophila melanogaster*),

5 AF068257 (*Drosophila melanogaster*), U50453 (*Thermus aquaticus*), U27343 (*Bacillus subtilis*), U71053 (U71053 (*Thermotoga maritima*), U71052 (*Aquifex pyrophilus*), U13696 (Human), U13695 (Human), M29687 (*S.typhimurium*), M63655 (*E. coli*) and L19346 (*Escherichia coli*). The nucleic acid molecules containing sequences of nucleotides

10 with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding MLH1 and in mutagenesis: AI389544 (*Drosophila melanogaster*), AI387992 (*Drosophila melanogaster*), AF068257 (*Drosophila melanogaster*), U80054 (*Rattus norvegicus*) and U07187 (*Saccharomyces cerevisiae*).

15 In a preferred embodiment, mutant *mutL* or MLH1 used in the present methods has a mutation in its catalytic site, ATP binding site or combination thereof (Ban and Yang, *Cell*, 95:541-552 (1998)).

In another preferred embodiment, the mutant *mutL* used in the present methods is an *E.Coli* mutant *mutL* having a E29K, E32K, A37T,

20 D58N, G60S, G93D, R95C, G96S, G96D, S112L, A16T, A16V, P305L, H308Y, G238D, S106F or A271V mutation (Aronshtam and Marinus, *Nucleic Acids Res.*, 24(13):2498-504 (1996)).

In still another preferred embodiment, the mutant MLH1 used in the present methods is a human mutant MLH1 having a P28L, M35R, S44F,

25 G67R, I68N, I107R, T117R, T117M, R265H, V185G or G224D mutation (Peltomaki and Vasen, *Gastroenterology*, 113(4):1146-58 (1997)).

d. **Mutant MutS**

In another specific embodiment, a mutant *mutS* is used in the present methods. The nucleic acid molecules containing sequences of

30 nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding *mutS* and in mutagenesis: AF146227

(Mus musculus), AF193018 (Arabidopsis thaliana), AF144608 (Vibrio parahaemolyticus), AF034759 (Homo sapiens), AF104243 (Homo sapiens), AF007553 (Thermus aquaticus caldophilus), AF109905 (Mus musculus), AF070079 (Homo sapiens), AF070071 (Homo sapiens),

5 AH006902 (Homo sapiens), AF048991 (Homo sapiens), AF048986 (Homo sapiens), U33117 (Thermus aquaticus), U16152 (Yersinia enterocolitica), AF000945 (Vibrio cholerae), U698873 (Escherichia coli), AF003252 (Haemophilus influenzae strain b (Eagan), AF003005 (Arabidopsis thaliana), AF002706 (Arabidopsis thaliana), L10319

10 (Mouse), D63810 (Thermus thermophilus), U27343 (Bacillus subtilis), U71155 (Thermotoga maritima), U71154 (Aquifex pyrophilus), U16303 (Salmonella typhimurium), U21011 (Mus musculus), M84170 (S. cerevisiae), M84169 (S. cerevisiae), M18965 (S. typhimurium) and M63007 (Azotobacter vinelandii).

15 Preferably, the mutant mutS used in the present methods has a mutation in its catalytic site, dimerization site, mutL interaction site or a combination thereof. Also preferably, the mutant mutS used in the present methods is an *E.Coli* mutant mutS (see, e.g., Wu *et al.*, *J. Biol. Chem.*, 274(9):5948-52 (1999)).

20 e. Mutant MutM

In still another specific embodiment, a mutant mutM is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding mutM and in mutagenesis: AF148219

25 (Nostoc PCC8009), AF026468 (Streptococcus mutans), AF093820 (Mastigocladus laminosus), AB010690 (Arabidopsis thaliana), U40620 (Streptococcus mutans), AB008520 (Thermus thermophilus) and AF026691 (Homo sapiens).

30 Preferably, the mutant mutM used in the present methods has a mutation in its catalytic site, mutY interaction site or combination thereof (Michaels *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 89(15):7022-5 (1992)).

Also preferably, the mutant mutM used in the present methods is an *E.Coli* mutant mutM having a K57G or K57R mutation (Sidorkina and Laval, *Nucleic Acids Res.*, 26(23):5351-7 (1998)).

f. **Mutant MutY**

5 In yet another specific embodiment, a mutant mutY is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding mutY and in mutagenesis: AF121797 (Streptomyces), U63329 (Human), AA409965 (Mus musculus) and  
10 AF056199 (Streptomyces).

Preferably, the mutant mutY used in the present methods has a mutation in its catalytic site, mutM interaction site or combination thereof (Michaels et al., *Proc. Natl. Acad. Sci. U.S.A.*, 89(15):7022-5 (1992)).

Also preferably, the mutant mutY used in the present methods is an  
15 *E.Coli* mutant mutY having an E37S, V45N, G116D, D138N or K142A mutation (Lu et al., *J. Biol. Chem.*, 271(39):24138-43 (1996); Guan et al., *Nat. Struct. Biol.*, 5(12):1058-64 (1998); and Wright et al., *J. Biol. Chem.*, 274(41):29011-18 (1999)). More preferably, the abnormal base-pairing to be detected is a A:C mismatch and the mutant DNA repair  
20 enzyme used in the present methods is a mutant MutY.

g. **Mutant uvrD**

In yet another specific embodiment, a mutant uvrD is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding uvrD and in mutagenesis: L02122 (*E. coli*), AF028736 (*Serratia marcescens*), AF010185 (*Pseudomonas aeruginosa*), D00069 (*Escherichia coli*), AB001291 (*Thermus thermophilus*), M38257 (*Escherichia coli*) and L22432 (*Mycoplasma capricolum*).

30 Preferably, the mutant uvrD used in the present methods has a mutation in its catalytic site, ATP binding site or combination thereof.

Also preferably, the mutant uvrD used in the present methods is an *E.Coli* mutant uvrD having a K35M, D220NE221Q, E221Q or Q251E mutation (Brosh and Matson, *J. Bacteriol.*, 177(19):5612-21 (1995); George et al., *J. Mol. Biol.*, 235(2):424-35 (1994); and Brosh and Matson, *J. Biol. Chem.*, 272(1):572-79 (1997)).

5       h.     **Mutant MSH2**

In yet another specific embodiment, a mutant MSH2 is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in 10 obtaining nucleic acid encoding MSH2 and in mutagenesis: AF109243 (Arabidopsis thaliana), AF030634 (Neurospora crassa), AF002706 (Arabidopsis thaliana), AF026549 (Arabidopsis thaliana), L47582 (Homo sapiens), L47583 (Homo sapiens), L47581 (Homo sapiens) and M84170 (*S. cerevisiae*).

15       Preferably, the mutant MSH2 used in the present methods has a mutation in its catalytic site, ATP binding site, ATPase site or combination thereof. Also preferably, the mutant MSH2 used in the present methods is a *S. cerevisiae* mutant MSH2 having a G693D or a G855D mutation (Alani et al., *Mol. Cell. Biol.*, 17(5):2436-47 (1997)), or 20 a human mutant MSH2 having a fragment encoding 195 amino acids within the C-terminal domain of hMSH-2 or having a K675R mutation (Whitehouse et al., *Biochem. Biophys. Res. Commun.*, 232(1):10-3 (1997); and Iaccarino et al., *EMBO J.*, 17(9):2677-86 (1998)).

17       i.     **Mutant MSH6**

25       In yet another specific embodiment, a mutant MSH6 is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding MSH6 and in mutagenesis: U54777 (Homo sapiens) and AF031087 (Mus musculus).

30       Preferably, the mutant MSH6 used in the present methods has a mutation in its catalytic site, ATP binding site, ATPase site or

combination thereof. Also preferably, the mutant MSH6 used in the present methods is a human mutant MSH6 having a K1140R mutation (Iaccarino et al., *EMBO J.*, 17(9):2677-86 (1998)). More preferably, the mutant DNA repair complex used in the present methods comprises a 5 human mutant MSH2 having a K675R mutation and a human mutant MSH6 having a K1140R mutation.

**j. Mutant T4 endonuclease V**

In yet another specific embodiment, a mutant T4 endonuclease V is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding T4 endonuclease V and in mutagenesis: M35392 (Synthetic), U76612 (Coliphage), U48703 (Bacteriophage T4) and M23414 (Synthetic). Preferably, the mutant T4 endonuclease V used in the present methods has a E23Q mutation (Doi et 15 al., *Proc. Natl. Acad. Sci. U.S.A.*, 89(20):9420-4 (1992)).

**k. Mutant MSH3**

In yet another specific embodiment, a mutant MSH3 is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in 20 obtaining nucleic acid encoding MSH3 and in mutagenesis: J04810 (Human) and M96250 (*Saccharomyces cerevisiae*).

**l. Mutant alkA**

In yet another specific embodiment, a mutant alkA is used in the present methods. The nucleic acid molecules containing sequences of 25 nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding alkA and in mutagenesis: D14465 (*Bacillus subtilis*) and K02498 (*E. coli*).

**m. Mutant Exonuclease I**

In yet another specific embodiment, a mutant exonuclease I is used 30 in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in

obtaining nucleic acid encoding exonuclease I and in mutagenesis:  
AF060479 (*Homo sapiens*), U86134 (*Saccharomyces cerevisiae*) and  
J02641 (*E. coli*).

n. **Mutant fen1**

5 In yet another specific embodiment, a mutant fen1 is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding fen1 and in mutagenesis: AF065397 (*Xenopus laevis* (FEN1)) and AF036327 (*Xenopus laevis* (FEN1)).

10 o. **Mutant rpa**

In yet another specific embodiment, a mutant rpa is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding rpa and in mutagenesis: AA955716 (Homo sapiens), AA955320 (Homo sapiens), AA925949 (Homo sapiens), U29383 (*Zea mays*), U33419 (Orf virus) and L07493 (Homo sapiens).

p. **Mutant pcna**

In yet another specific embodiment, a mutant pcna is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding pcna and in mutagenesis: AB025029 (*Nicotiana tabacum*), AF038875 (*Nicotiana tabacum*), AF104412 (*Nicotiana tabacum*), AA925316 (*Rattus norvegicus*), AA924358 (*Rattus norvegicus*), AA923907 (*Rattus norvegicus*), AA901212 (*Rattus norvegicus*), AA858643 (*Rattus norvegicus*), AA441366 (*Drosophila melanogaster*), AA440162 (*Drosophila melanogaster*), L42763 (*Styela clava*), AF085197 (*Nicotiana tabacum*), AF020427 (*Sarcophaga crassipalpis*), AB002264 (*Bombyx mori*), J04718 (Human), M34080 (*X.laevis*) and M33950 (*D.melanogaster*).

q. **Mutant Replication factor C**

In yet another specific embodiment, a mutant replication factor C is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding replication factor C and in

5 mutagenesis: AF139987 (*Mus musculus*), AA924760 (*Homo sapiens*), AA901331 (*Homo sapiens*), AA900852 (*Homo sapiens*), AA899302 (*Homo sapiens*), AA819500 (*Rattus norvegicus*), U60144 (*Anas platyrhynchos*), U26031 (*Saccharomyces cerevisiae*), U26030 (*Saccharomyces cerevisiae*), U26029 (*Saccharomyces cerevisiae*),

10 U26028 (*Saccharomyces cerevisiae*), U26027 (*Saccharomyces cerevisiae*), AF045555 (*Homo sapiens*), U86620 (*Emericella nidulans*), U86619 (*Emericella nidulans*), D28499 (Yeast), U07685 (*Drosophila melanogaster*), M87338 (Human), M87339 (Human), L07540 (Human), L07541 (Human), L20502 (*Saccharomyces cerevisiae*), L18755

15 (Saccharomyces cerevisiae), U12438 (*Gallus gallus Leghorn*) and L23320 (Human).

r. Mutant Uracil DNA glycosylase

In yet another specific embodiment, a mutant uracil DNA glycosylase (UDG) is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding uracil DNA glycosylase and in mutagenesis: AF174292 (*Schizosaccharomyces pombe*), AF108378 (*Cercopithecine herpesvirus*), AF125182 (*Homo sapiens*), AF125181 (*Xenopus laevis*), U55041 (*Homo sapiens*), U55041 (*Mus musculus*), AF084182 (Guinea pig cytomegalovirus), U31857 (Bovine herpesvirus), AF022391 (Feline herpesvirus), M87499 (Human), J04434 (Bacteriophage PBS2), U13194 (Human herpesvirus 6), L34064 (Gallid herpesvirus 1), U04994 (Gallid herpesvirus 2), L01417 (Rabbit fibroma virus), M25410 (Herpes simplex virus type 2), J04470 (*S.cerevisiae*), J03725 (*E.coli*), U02513 (Suid herpesvirus), U02512 (Suid herpesvirus) and L13855 (Pseudorabies

virus).

s. **Mutant Thymidine DNA glycosylase**

In yet another specific embodiment, a mutant thymidine DNA glycosylase (TDG) is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding thymidine DNA glycosylase and in mutagenesis: AF117602 (*Ateles paniscus chamek*). Preferably, the abnormal base-pairing to be detected is a G:T mismatch and the mutant DNA repair enzyme used in the present methods is a mutant TDG (Hsu et al., *Carcinogenesis*, 15(8):1657-62 (1994)).

t. **Mutant dam**

In yet another specific embodiment, a mutant dam is used in the present methods. The nucleic acid molecules containing sequences of nucleotides with the following GenBank accession Nos. can be used in obtaining nucleic acid encoding dam and in mutagenesis: AF091142 (*Neisseria meningitidis* strain BF13), AF006263 (*Treponema pallidum*), U76993 (*Salmonella typhimurium*) and M22342 (Bacteriophage T2).

**2. Detecting the binding of the mutant enzyme**

Binding of the mutant enzyme to a duplex can be detected by any method known to those of skill in the art for detection of proteins. The enzyme may be specifically labeled, such as with a fluorescent label, radiolabeled, tagged with a readily tag that can be readily purified, labeled with another enzyme, or antibody. In an exemplary embodiment, biotin is bound to the mutant enzyme, which can then interact with a streptavidin-labeled moiety, such as horse radish peroxidase (HRPO), which upon reaction with an appropriate substrate will form a colored product.

For example, an array of nucleic acid probes, containing for example, from about 20 to about 50 up to about 100 nucleotides, are hybridized with single-stranded nucleic acid from a sample. The hybrids

are contacted with a selected or a plurality of mutant enzymes, which are labeled with biotin. After contacting the biotin reacts with streptavidin which is labeled, such as with HRPO, and the bound mutant enzyme is detected by virtue of the formation of detectable product, such as colored product. If the probes on the array are of known sequence, selected, for example for inclusion of polymorphisms, then upon reaction, the presence or absence of an array of polymorphism in the sample can be rapidly and readily identified.

10       **C. METHODS FOR DETECTING MUTATIONS IN NUCLEIC ACIDS FOR PROGNOSIS AND DIAGNOSIS OF DISEASES, DISORDERS AND INFECTIONS**

Also provided herein are methods for detecting a mutations in a nucleic acid molecule for diagnostic and prognostic applications. These methods involve binding a mutant nucleic acid binding enzyme, such as a 15 mutant repair enzyme to nucleic acids in sample, such as body tissue or fluid sample, and detecting the bound mutant enzyme. These reactions can be performed in solution, or, preferably in solid phase.

In one embodiment, single-stranded nucleic acids, either those known to be wild type or with a mutation indicative of a particular disorder are hybridized with the sample nucleic acid. The resulting duplexes are contacted with a selected mutant enzyme or a plurality thereof that contain different specificities. The resulting complexes, which are indicative a difference in sequence between the strands in the sample from the known strands, are detected. These methods can be 25 performed in solution or preferably in solid phase. In a preferred embodiment, the single-stranded nucleic acids containing known sequences are on the solid support. In others, the enzymes of known specificities can be bound on a solid support. Bound hybrids are indicative of the mutation present.

30       In a preferred embodiment, the method is performed by hybridizing a strand of a nucleic acid having or suspected of having a mutation with

a complementary strand of a wild-type nucleic acid (or with a strand having a known mutation), whereby the mutation results in an abnormal base-pairing in the formed nucleic acid duplex; contacting the nucleic acid duplex with a mutant DNA repair enzyme or complex thereof, where the

5 mutant DNA repair enzyme or complex thereof has binding affinity for the abnormal base-pairing in the duplex but has attenuated catalytic activity; and detecting binding between the nucleic acid duplex and the mutant DNA repair enzyme or complex thereof, whereby the presence or quantity of the mutation is assessed.

10 Any mutant DNA repair enzymes or complexes thereof that have binding affinity for the abnormal base-pairing in the duplex but have attenuated catalytic activity can be used in the mutation detection. Preferably, the mutant DNA repair enzymes or complexes thereof described in the above Section B can be used. Typically, the nucleic

15 acid strand to be tested and the complementary wild-type nucleic acid strand are DNA strands.

Mutations that can be detected by these methods, include those that are associated with or that are indicative of a disease or disorder or predilection thereto, or infection by a pathological agent. These methods

20 can be used for prognosis or diagnosis of the presence or severity of the disease, disorder or infection.

Any diseases, disorders or infections that are associated with a nucleic acid mutation or for which such mutation serves as a marker or indicator can be diagnosed or the tendency therefor prognosticated using

25 the present methods. Such diseases and disorders include, but are not limited to, cancers, immune system diseases or disorders, metabolism diseases or disorders, muscle and bone diseases or disorders, nervous system diseases or disorders, signal diseases or disorders and transporter diseases or disorders. Infections include, but are not limited to, infections

30 caused by viruses, eubacteria, archaebacteria and eukaryotic pathogens.

Among the diseases or disorders that can be diagnosed or the

tendency to develop them, include but are not limited to, a disease or disorder associated with an androgen receptor mutation, tetrahydrobiopterin deficiencies, X-Linked agammaglobulinemia, a disease or disorder associated with a factor VII mutation, anemia, a disease or disorder  
5 associated with a glucose-6-phosphate mutation, the glycogen storage disease type II (Pompe Disease), hemophilia A, a disease or disorder associated with a hexosaminidase A mutation, a disease or disorder associated with a human type I or type III collagen mutation, a disease or disorder associated with a rhodopsin or RDS mutation, a disease or  
10 disorder associated with a L1CAM mutation, a disease or disorder associated with a LDL receptor mutation, a disease or disorder associated with an ornithine transcarbamylase mutation, a disease or disorder associated with a PAX6 mutation and a disease or disorder associated with a von Willebrand factor mutation.

15        1.     **Cancer**

Any cancers that are associated with a mutation(s) in a nucleic acid can be predicted or diagnosed using the present methods. For example, breast cancer, Burkitt lymphoma, colon cancer, small cell lung carcinoma, melanoma, multiple endocrine neoplasia (MEN),  
20 neurofibromatosis, p53-associated tumor, pancreatic carcinoma, prostate cancer, Ras-associated tumor, retinoblastoma and Von-Hippel Lindau disease (VHL) can be predicted or diagnosed using the present methods.

a.     **Breast cancer**

Two breast cancer susceptibility genes have been identified:  
25 BRCA1 on chromosome 17 and BRCA2 on chromosome 13. When an individual carries a mutation in either BRCA1 or BRCA2, they are at an increased risk of being diagnosed with breast or ovarian cancer at some point in their lives (Albertsen et al., *Am. J. Hum. Genet.*, 54(3):516-25 (1994); and Wooster et al., *Nature*, 378(6559):789-92 (1995)). Until  
30 recently, it was not clear what the function of these genes was, until studies on a related protein in yeast revealed their normal role: they

participate in repairing radiation-induced breaks in double-stranded DNA. It is thought that mutations in BRCA1 or BRCA2 might disable this mechanism, leading to more errors in DNA replication and ultimately to cancerous growth.

5 In a specific embodiment, the breast cancer to be predicted or diagnosed according to the present method is associated with a mutation in BRCA1 or BRCA2.

b. Burkitt lymphoma

Burkitt lymphoma results from chromosome translocations that involve the Myc gene. A chromosome translocation means that a chromosome is broken, which allows it to associate with parts of other chromosomes (Adams et al., *Proc. Natl. Acad. Sci. U.S.A.*, 80(7):1982-6 (1983); Watt et al., *Nature*, 303(5919):725-8 (1983); and Cole, *Annu. Rev. Genet.*, 20:361-84 (1986)). The classic chromosome translocation in Burkitt lymphoma involves chromosome 8, the site of the Myc gene. This changes the pattern of Myc's expression, thereby disrupting its usual function in controlling cell growth and proliferation.

In a specific embodiment, the Burkitt lymphoma to be predicted or diagnosed according to the present method is associated with a mutation in Myc.

c. Colon cancer

Colon cancer is one of the most common inherited cancer syndromes known. Two key genes involved in colon cancer have been found: MSH2, on chromosome 2 and MLH1, on chromosome 3.

25 Normally, the protein products of these genes help to repair mistakes made in DNA replication. If the MSH2 and MLH1 proteins are mutated and therefore don't work properly, the replication mistakes are not repaired, leading to damaged DNA and, in this case, colon cancer (Bronner et.al., *Nature*, 368(6468):258-61 (1994); and Fishel et al., *Cell*, 75(5):1027-38 (1993)).

In a specific embodiment, the colon cancer to be predicted or

diagnosed according to the present method is associated with a mutation in MSH2 or MLH1.

d. **Small cell lung carcinoma**

Small cell lung carcinoma is distinctive from other kinds of lung cancer (metastases are already present at the time of discovery) and accounts for approximately 110,000 cancer diagnoses annually. A deletion of part of chromosome 3, SCLC1, was first observed in 1982 in small cell lung carcinoma cell lines (Whang-Peng et al., *Science*, 215(4529):181-2 (1982)).

10 In a specific embodiment, the small cell lung carcinoma to be predicted or diagnosed according to the present method is associated with a mutation in SCLC1.

e. **Melanoma carcinoma**

In some cases, the risk of developing melanoma runs in families, where a mutation in the CDKN2 gene on chromosome 9 can underlie susceptibility to melanoma (Hussussian et al., *Nat. Genet.*, 8(1):15-21 (1994)). CDKN2 codes for a protein called p16 that is an important regulator of the cell division cycle: it stops the cell from synthesizing DNA before it divides. If p16 is not working properly, the skin cell does not have this brake on the cell division cycle, and so can go on to proliferate unchecked. At some point this proliferation can be seen as a sudden change in skin growth or the appearance of a mole.

20 In a specific embodiment, the melanoma carcinoma to be predicted or diagnosed according to the present method is associated with a mutation in CDKN2.

f. **Multiple endocrine neoplasia**

Multiple endocrine neoplasia (MEN) is a group of rare diseases caused by genetic defects that lead to hyperplasia (abnormal multiplication or increase in the number of normal cells in normal arrangement in a tissue) and hyperfunction (excessive functioning) of 2 or more components of the endocrine system. Normally, the hormones

released by endocrine glands are carefully balanced to meet the body's needs. When a person has MEN, specific endocrine glands, such as the parathyroid glands, the pancreas gland and the pituitary gland, tend to become overactive. When these glands go into overdrive, the result can

5 be: excessive calcium in the bloodstream (resulting in kidney stones or kidney damage); fatigue; weakness; muscle or bone pain; constipation; indigestion; and thinning of bones. The MEN1 gene, which has been known for several years to be found on chromosome 11, was more finely mapped in 1997 (Chandrasekharappa et al., *Science*, 276(5311):404-7

10 (1997)). In a specific embodiment, the MEN to be diagnosed or predicted according to the present method is associated with a mutation in MEN1.

g. **Neurofibromatosis**

Neurofibromatosis, type 2 (NF-2), is a rare inherited disorder

15 characterized by the development of benign tumors on auditory nerves (acoustic neuromas). The disease is also characterized by the development of malignant central nervous system tumors as well. The NF2 gene has been mapped to chromosome 22 and is thought to be a 'tumor-suppressor gene' (Rouleau et al., *Nature*, 363(6429):515-21

20 (1993)). A mutation in NF2 impairs its function, and accounts for the clinical symptoms observed in neurofibromatosis sufferers. NF-2 is an autosomal dominant genetic trait; it affects both genders equally and each child of an affected parent has a 50% chance of inheriting the gene.

In a specific embodiment, the neurofibromatosis to be predicted or

25 diagnosed according to the present method is associated with a mutation in NF2.

h. **Cancer associated with p53 mutation**

The p53 gene is a tumor suppressor gene (Harlow et al., *Mol. Cell. Biol.*, 5(7):1601-10 (1985)). If a person inherits only one functional copy

30 of the p53 gene from their parents, they are predisposed to cancer and usually develop several independent tumors in a variety of tissues in early

adulthood. This condition is rare, and is known as Li-Fraumeni syndrome. Mutations in p53 are found in most tumor types, and so contribute to the complex network of molecular events leading to tumor formation. The p53 gene has been mapped to chromosome 17. In the cell, p53 protein binds DNA, which in turn stimulates another gene to produce a protein called p21 that interacts with a cell division-stimulating protein (cdk2). When p21 is complexed with cdk2 the cell cannot pass through to the next stage of cell division. Mutant p53 can no longer bind DNA in an effective way, and as a consequence the p21 protein is not made available to act as the 'stop signal' for cell division. Thus cells divide uncontrollably, and form tumors.

In a specific embodiment, the cancer to be predicted or diagnosed according to the present method is associated with a mutation in p53.

i. **Pancreatic carcinoma**

About 90% of human pancreatic carcinomas show a loss of part of chromosome 18. In 1996, a possible tumor suppressor gene, DPC4 (Smad4), was discovered from the section that is lost in pancreatic cancer, so may play a role in pancreatic cancer (Hahn et al., *Science*, 271(5247):350-3 (1996)). There is a whole family of Smad proteins in vertebrates, all involved in signal transduction of transforming growth factor-beta (TGF-beta) related pathways.

In a specific embodiment, the pancreatic carcinoma to be predicted or diagnosed according to the present method is associated with a mutation in DPC4 (Smad4).

25 j. **Prostate cancer**

One of the most promising recent breakthroughs in prostate cancer research is the discovery of a susceptibility locus for prostate cancer on chromosome 1, called HPC1, which may account for about 1 in 500 cases of prostate cancer (Smith et al., *Science*, 274(5291):1371-4 (1996)).

In a specific embodiment, the prostate cancer to be predicted or

diagnosed according to the present method is associated with a mutation in HPC1.

k. **Cancer associated with Ras oncogene**

Ras is an oncogene product that is found on chromosome 11. It is

5 found in normal cells, where it helps to relay signals by acting as a switch (Lowy and Willumsen, *Annu. Rev. Biochem.*, 62:851-91 (1993); Russell et al., *Genomics*, 35(2):353-60 (1996); and Tong et al., *Nature*, 337(6202):90-3 (1989)). When receptors on the cell surface are stimulated (by a hormone, for example), Ras is switched on and

10 transduces signals that tell the cell to grow. If the cell-surface receptor is not stimulated, Ras is not activated and so the pathway that results in cell growth is not initiated. In about 30% of human cancers, Ras is mutated so that it is permanently switched on, telling the cell to grow regardless of whether receptors on the cell surface are activated or not.

15 In a specific embodiment, the cancer to be predicted or diagnosed according to the present method is associated with a mutation in Ras oncogene.

l. **Retinoblastoma**

Retinoblastoma occurs in early childhood and develops from the

20 immature retina - the part of the eye responsible for detecting light and color. There are hereditary and non-hereditary forms of retinoblastoma. In the hereditary form, multiple tumors are found in both eyes, while in the non-hereditary form only one eye is effected and by only one tumor. In the hereditary form, a gene called Rb is lost from chromosome 13

25 (Friend et al., *Nature*, 323(6089):643-6 (1986); and Lee et al., *Science*, 235(4794):1394-9 (1987)). Rb is found in all cells of the body, where under normal conditions it acts as a brake on the cell division cycle by preventing certain regulatory proteins from triggering DNA replication. If Rb is missing, a cell can replicate itself over and over in an uncontrolled

30 manner, resulting in tumor formation.

In a specific embodiment, the retinoblastoma to be predicted or

diagnosed according to the present method is associated with a mutation in Rb gene.

**m. Von-Hippel Lindau syndrome**

Von-Hippel Lindau syndrome is an inherited multi-system disorder

5 characterized by abnormal growth of blood vessels. While blood vessels normally grow like trees, in people with VHL little knots of blood capillaries sometimes occur. These knots are called angiomas or hemangioblastomas. Growths may develop in the retina, certain areas of the brain, the spinal cord, the adrenal glands and other parts of the body.

10 The gene for Von-Hippel Lindau disease (VHL) is found on chromosome 3, and is inherited in a dominant fashion (Latif et al., *Science*, 260(5112):1317-20 (1993)). If one parent has a dominant gene, each child has a 50-50 chance of inheriting that gene. The VHL gene is a tumor suppressor gene.

15 In a specific embodiment, the Von-Hippel Lindau syndrome to be predicted or diagnosed according to the present method is associated with a mutation in VHL gene.

**2. Immune system disease or disorder**

Any immune system diseases or disorders that are associated with

20 a mutation(s) in a nucleic acid can be predicted or diagnosed using the present methods. For example, autoimmune polyglandular syndrome type I (APS1, also called APECED), inflammatory bowel disease (IBD), DiGeorge syndrome, familial Mediterranean fever (FMF) and severe combined immunodeficiency (SCID) can be predicted or diagnosed using

25 the present methods.

**a. Autoimmune polyglandular syndrome type I**

Autoimmune polyglandular syndrome type I (APS1, also called APECED) is a rare autosomal recessive disorder that maps to human chromosome 21. At the end of 1997, researchers reported that they

30 isolated a novel gene, which they called AIRE (autoimmune regulator). Database searches revealed that the protein product of this gene is a

transcription factor - a protein that plays a role in the regulation of gene expression. The researchers showed that mutations in this gene are responsible for the pathogenesis of APS1 (Nagamine et al., Nat Genet., 17(4):393-8 (1997)).

5 In a specific embodiment, the autoimmune polyglandular syndrome type I to be predicted or diagnosed according to the present method is associated with a mutation in AIRE gene.

b. Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a group of chronic disorders 10 that cause inflammation or ulceration in the small and large intestines. Most often, IBD is classified either as ulcerative colitis or Crohn disease. While ulcerative colitis affects the inner lining of the colon and rectum, Crohn disease extends into the deeper layers of the intestinal wall. It is a chronic condition and may recur at various times over a lifetime. About 15 20% of cases of Crohn disease appear to run in families. It is a 'complex trait', which means that several genes at different locations in the genome may contribute to the disease. A susceptibility locus for the disease was recently mapped to chromosome 16. Candidate genes found in this region include several involved in the inflammatory response, 20 including: CD19, involved in B-lymphocyte function; sialophorin, involved in leukocyte adhesion; the CD11 integrin cluster, involved in microbacteria cell adhesion; and the interleukin-4 receptor, which is interesting, as IL-4-mediated functions are altered in IBDs (Hugot et al., *Nature*, 379(6568):821-3 (1996)).

25 In a specific embodiment, the inflammatory bowel disease to be predicted or diagnosed according to the present method is associated with a mutation in CD19, sialophorin, CD11 integrin cluster or interleukin-4 receptor.

c. DiGeorge syndrome

30 DiGeorge syndrome is a rare congenital (*i.e.*, present at birth) disease whose symptoms vary greatly between individuals, but

commonly include a history of recurrent infection, heart defects and characteristic facial features. DiGeorge syndrome is caused by a large deletion from chromosome 22, produced by an error in recombination at meiosis (the process that creates germ cells and ensures genetic variation 5 in the offspring). This deletion means that several genes from this region are not present in DiGeorge syndrome patients. It appears that the variation in the symptoms of the disease is related to the amount of genetic material lost in the chromosomal deletion (Budarf et al., *Nat. Genet.*, 10(3):269-78 (1995)).

10           d.     **Familial Mediterranean fever**

Familial Mediterranean fever (FMF) is an inherited disorder usually characterized by recurrent episodes of fever and peritonitis (inflammation of the abdominal membrane). In 1997, researchers identified the gene for FMF and found several different gene mutations that cause this inherited 15 rheumatic disease. The gene, found on chromosome 16, codes for a protein that is found almost exclusively in granulocytes - white blood cells important in the immune response. The protein is likely to normally assist in keeping inflammation under control by deactivating the immune response - without this 'brake', an inappropriate full-blown inflammatory 20 reaction occurs: an attack of FMF (*Cell*, 90(4):797-807 (1997); and *Nat. Genet.*, 17(1):25-31 (1997)).

In a specific embodiment, the familial Mediterranean fever to be predicted or diagnosed according to the present method is associated with a mutation in FMF gene.

25           e.     **Severe combined immunodeficiency**

Severe combined immunodeficiency (SCID) represents a group of rare, sometimes fatal, congenital disorders characterized by little or no immune response (Valerio et al., *EMBO J.*, 4(2):437-43 (1985); and Noguchi et al., *Cell*, 73(1):147-57 (1993)). The defining feature of SCID, 30 commonly known as "bubble boy" disease, is a defect in the specialized white blood cells (B- and T-lymphocytes) that defend us from infection by

viruses, bacteria and fungi. Without a functional immune system, SCID patients are susceptible to recurrent infections such as pneumonia, meningitis and chicken pox, and can die before the first year of life. All forms of SCID are inherited, with as many as half of SCID cases linked to

5 the X chromosome, passed on by the mother. X-linked SCID results from a mutation in the interleukin 2 receptor gamma (IL2RG) gene which produces the common gamma chain subunit, a component of several IL receptors. Defective IL receptors prevent the proper development of T-lymphocytes that play a key role in identifying invading agents as well as

10 activating and regulating other cells of the immune system. In another form of SCID, there is a lack of the enzyme adenosine deaminase (ADA), coded for by a gene on chromosome 20. This means that the substrates for this enzyme accumulate in cells. Immature lymphoid cells of the immune system are particularly sensitive to the toxic effects of these

15 unused substrates, so fail to reach maturity. As a result, the immune system of the afflicted individual is severely compromised or completely lacking.

In a specific embodiment, the severe combined immunodeficiency to be predicted or diagnosed according to the present method is

20 associated with a mutation in interleukin 2 receptor gamma (IL2RG) or adenosine deaminase (ADA).

### 3. Metabolism system diseases and disorders

Any metabolism diseases or disorders that are associated with a mutation(s) in a nucleic acid can be predicated or diagnosed using the

25 present methods. For example, adrenoleukodystrophy (ALD), atherosclerosis, Gaucher disease, gyrate atrophy of the choroid, diabetes, obesity, paroxysmal nocturnal hemoglobinuria (PNH), phenylketonuria (PKU), Refsum disease and Tangier disease (TD) can be predicted or diagnosed using the present methods.

#### 30 a. Adrenoleukodystrophy

Adrenoleukodystrophy (ALD) is a rare, inherited metabolic disorder.

In this disease the fatty covering (myelin sheath) on nerve fibers in the brain is lost, and the adrenal gland degenerates, leading to progressive neurological disability and death. People with ALD accumulate high levels of saturated, very long chain fatty acids in their brain and adrenal cortex because the fatty acids are not broken down by an enzyme in the normal manner. So, when the ALD gene was discovered in 1993, it was a surprise that the corresponding protein was in fact a member of a family of transporter proteins, not an enzyme (Mosser et al., *Nature*, 361(6414):726-30 (1993)).

10 In a specific embodiment, the adrenoleukodystrophy to be predicted or diagnosed according to the present method is associated with a mutation in ALD gene.

b. **Atherosclerosis**

Atherosclerosis is characterized by a narrowing of the arteries caused by cholesterol-rich plaques of immune-system cells. Key risk factors for atherosclerosis, which can be genetic and/or environmental, include: elevated levels of cholesterol and triglyceride in the blood, high blood pressure and cigarette smoke. A protein called apolipoprotein E, which can exist in several different forms, is coded for by a gene found on chromosome 19. It is important for removing excess cholesterol from the blood, and does so by carrying cholesterol to receptors on the surface of liver cells. Defects in apolipoprotein E sometimes result in its inability to bind to the receptors, which leads to an increase a person's blood cholesterol, and consequently their risk of atherosclerosis (Das et al., *J. Biol. Chem.*, 260(10):6240-7 (1985); and Breslow, *Science*, 272(5262):685-8 (1996)).

In a specific embodiment, the atherosclerosis to be predicted or diagnosed according to the present method is associated with a mutation in apolipoprotein E.

30 c. **Gaucher disease**

Gaucher disease is an inherited illness caused by a gene mutation

(Barneveld et al., *Hum. Genet.*, 64(3):227-31 (1983); and Beutler, *Science*, 256(5058):794-9 (1992)). Normally, this gene is responsible for an enzyme called glucocerebrosidase that the body needs to break down a particular kind of fat called glucocerebroside. In people with

5 Gaucher disease, the body is not able to properly produce this enzyme and the fat cannot be broken down. It then accumulates, mostly in the liver, spleen and bone marrow. Gaucher disease can result in pain, fatigue, jaundice, bone damage, anemia and even death.

In a specific embodiment, the Gaucher disease to be predicted or

10 diagnosed according to the present method is associated with a mutation in glucocerebrosidase.

d. Gyrate atrophy of the choroid

People suffering from gyrate atrophy of the choroid (the thin coating of the eye) and retina face a progressive loss of vision, with total

15 blindness usually occurring between the ages of 40 and 60. The disease is an inborn error of metabolism. The gene whose mutation causes gyrate atrophy is found on chromosome 10, and encodes an enzyme called ornithine ketoacid aminotransferase (OAT) (Akaki et al., *J. Biol. Chem.*, 267(18):12950-4 (1992); and O'Donnell et al., *Am. J. Hum.*

20 *Genet.*, 43(6):922-8 (1988)). Different inherited mutations in OAT cause differences in the severity of symptoms of the disease. OAT converts the amino acid ornithine from the urea cycle ultimately into glutamate. In gyrate atrophy, where OAT function is affected, there is an increase in plasma levels of ornithine.

25 In a specific embodiment, the gyrate atrophy of the choroid to be predicted or diagnosed according to the present method is associated with a mutation in ornithine ketoacid aminotransferase (OAT).

e. Diabetes

Diabetes is a chronic metabolic disorder that adversely affects the

30 body's ability to manufacture and use insulin, a hormone necessary for the conversion of food into energy. The disease greatly increases the risk

of blindness, heart disease, kidney failure, neurological disease and other conditions for the approximately 16 million Americans who are affected by it. Type I, or juvenile onset diabetes, is the more severe form of the illness. Type I diabetes is what is known as a 'complex trait', which

5 means that mutations in several genes likely contribute to the disease (Nuffield et al., *Nature*, 371(6493):130-6 (1994)). For example, it is now known that the insulin-dependent diabetes mellitus (IDDM1) locus on chromosome 6 may harbor at least one susceptibility gene for Type I diabetes. In Type I diabetes, the body's immune system mounts an

10 immunological assault on its own insulin and the pancreatic cells that manufacture it. About 10 loci in the human genome have now been found that seem to confer susceptibility to Type I diabetes. Among these are (1) a gene at the locus IDDM2 on chromosome 11 and (2) the gene for glucokinase (GCK), an enzyme that is key to glucose metabolism

15 which helps modulate insulin secretion, on chromosome 7.

In a specific embodiment, the diabetes of the choroid to be predicted or diagnosed according to the present method is associated with a mutation in insulin-dependent diabetes mellitus (IDDM1) locus, a gene at the locus IDDM2, or glucokinase (GCK).

20

f.      **Obesity**

Obesity is an excess of body fat that frequently results in a significant impairment of health. Evidence suggests that obesity has

25 more than one cause: genetic, environmental, psychological and other factors may all play a part. The hormone leptin, produced by adipocytes (fat cells), was discovered about three years ago in mice (Zhang et al., *Nature*, 372(6505):425-32 (1994)). Subsequently the human Ob gene was mapped to chromosome 7. Leptin is thought to act as a lipostat: as

30 the amount of fat stored in adipocytes rises, leptin is released into the blood and signals to the brain that the body has enough to eat. Most

overweight people have high levels of leptin in their bloodstream, indicating that other molecules also effect feelings of salty and contribute to the regulation of body weight.

In a specific embodiment, the obesity to be predicted or diagnosed according to the present method is associated with a mutation in leptin or human Ob gene.

g. **Paroxysmal nocturnal hemoglobinuria**

The paroxysmal nocturnal hemoglobinuria (PNH) is characterized by a decreased number of red blood cells (anemia), and the presence of blood in the urine (hemoglobinuria) and plasma (hemoglobinemia), which is evident after sleeping. PNH is associated with a high risk of major thrombotic events, most commonly thrombosis of large intra-abdominal veins. Most patients who die of their disease die of thrombosis. PNH blood cells are deficient in an enzyme known as PIG-A, which is required for the biosynthesis of cellular anchors (Bessler et al., *EMBO J.*, 13(1):110-7 (1994); and Miyata et al., *Science*, 259(5099):1318-20 (1993)). Proteins that are partly on the outside of cells are often attached to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor, and PIG-A is required for the synthesis of a key anchor component. If PIG-A is defective, surface proteins that protect the cell from destructive components in the blood (complement) are not anchored and therefore absent, so the blood cells are broken down. The PIG-A gene is found on the X chromosome. Although not an inherited disease, PNH is a genetic disorder, known as an acquired genetic disorder. The affected blood cell clone passes the altered PIG-A to all its descendants--red cells, leukocytes (including lymphocytes), and platelets. The proportion of abnormal red blood cells in the blood determines the severity of the disease.

In a specific embodiment, the paroxysmal nocturnal hemoglobinuria to be predicted or diagnosed according to the present method is associated with a mutation in PIG-A.

h. **Phenylketonuria**

Phenylketonuria (PKU) is an inherited error of metabolism caused by a deficiency in the enzyme phenylalanine hydroxylase (DiLella et al., *Nature*, 327(6120):333-6 (1987); and Kwok et al., *Biochemistry*, 5 24(3):556-61 (1985)). Loss of this enzyme results in mental retardation, organ damage, unusual posture and can, in cases of maternal PKU, severely compromise pregnancy. Classical PKU is an autosomal recessive disorder, caused by mutations in both alleles of the gene for phenylalanine hydroxylase (PAH), found on chromosome 12. In the body, 10 phenylalanine hydroxylase converts the amino acid phenylalanine to tyrosine, another amino acid. Mutations in both copies of the gene for PAH means that the enzyme is inactive or is less efficient, and the concentration of phenylalanine in the body can build up to toxic levels. In some cases, mutations in PAH will result in a phenotypically mild form of 15 PKU called hyperphenylalanemia. Both diseases are the result of a variety of mutations in the PAH locus; in those cases where a patient is heterozygous for two mutations of PAH (ie each copy of the gene has a different mutation), the milder mutation will predominate.

In a specific embodiment, the phenylketonuria to be predicted or 20 diagnosed according to the present method is associated with a mutation in phenylalanine hydroxylase.

i. **Refsum disease**

Refsum disease is a rare disorder of lipid metabolism that is inherited as a recessive trait. Symptoms may include a degenerative 25 nerve disease (peripheral neuropathy), failure of muscle coordination (ataxia), retinitis pigmentosa (a progressive vision disorder), and bone and skin changes. Refsum disease is characterized by an accumulation of phytanic acid in the plasma and tissues. is a derivative of phytol; a component of chlorophyll. In 1997 the gene for Refsum disease was 30 identified and mapped to chromosome 10 (Jansen et al., *Nat. Genet.*, 17(2):190-3 (1997); and Mihalik et al., *Nat. Genet.*, 17(2):185-9 (1997)).

The protein product of the gene, PAHX, is an enzyme that is required for the metabolism of phytanic acid. Refsum disease patients have impaired PAHX - phytanic acid hydrolase.

In a specific embodiment, the Refsum disease to be predicted or

5 diagnosed according to the present method is associated with a mutation in PAHX.

j. Tangier disease

Tangier disease (TD) is a genetic disorder of cholesterol transport named for the secluded island of Tangier, located off the coast of

10 Virginia. TD was first identified in a five-year-old inhabitant of the island who had characteristic orange tonsils, very low levels of high density lipoprotein (HDL) or 'good cholesterol', and an enlarged liver and spleen. TD is caused by mutations in the *ABC1* (ATP-binding cassette) gene on chromosome 9q31 (Rust et al., *Nat. Genet.*, 22(4):352-5 (1999);

15 Bodzioch et al., *Nat. Genet.*, 22(4):347-51 (1999); Brooks-Wilson et al., *Nat. Genet.*, 22(4):336-45 (1999); and Rust et al., *Nat. Genet.*, 20(1):96-8 (1998)). *ABC1* codes for a protein that helps rid cells of excess cholesterol. This cholesterol is then picked up by HDL particles in the blood and carried to the liver, which processes the cholesterol to be

20 reused in cells throughout the body. Individuals with TD are unable to eliminate cholesterol from cells, leading to its buildup in the tonsils and other organs.

In a specific embodiment, the Tangier disease to be predicted or

diagnosed according to the present method is associated with a mutation

25 in *ABC1* (ATP-binding cassette) gene on chromosome 9q31.

4. Muscle and bone diseases and disorders

Any muscle and bone diseases or disorders that are associated with a mutation(s) in a nucleic acid can be predicted or diagnosed using the present methods. For example, Duchenne muscular dystrophy

30 (DMD), ELLIS-VAN CREVELD syndrome (chondroectodermal dysplasia), Marfan syndrome and myotonic dystrophy can be predicted or diagnosed

using the present methods.

a. **Duchenne muscular dystrophy**

Duchenne muscular dystrophy (DMD) is one of a group of muscular dystrophies characterized by the enlargement of muscles. The gene for 5 DMD, found on the X chromosome, encodes a large protein - dystrophin (Koenig et al., *Cell*, 53(2):219-26 (1988)). Dystrophin is required inside muscle cells for structural support: it is thought to strengthen muscle cells by anchoring elements of the internal cytoskeleton to the surface membrane. Without it, the cell membrane becomes permeable, so that 10 extracellular components enter the cell, increasing the internal pressure until the muscle cell 'explodes' and dies. The subsequent immune response can add to the damage.

In a specific embodiment, the Duchenne muscular dystrophy to be predicted or diagnosed according to the present method is associated 15 with a mutation in dystrophin.

b. **Ellis-Van Creveld syndrome**

Ellis-Van Creveld syndrome, also known as 'chondroectodermal dysplasia', is a rare genetic disorder characterized by short-limb dwarfism, polydactyly (additional fingers or toes), malformation of the bones of the 20 wrist, dystrophy of the fingernails, partial hare-lip, cardiac malformation and often prenatal eruption of the teeth. The gene causing Ellis-van Creveld syndrome, EVC, has been mapped to the short arm of chromosome 4 (Polymeropoulos et al., *Genomics*, 35(1):1-5 (1996)). A pattern of inheritance can be observed that has indicated the disease is 25 autosomal-recessive (*i.e.*, a mutated gene from both parents is required before the effects of the disease to become apparent).

In a specific embodiment, the Ellis-Van Creveld syndrome to be predicted or diagnosed according to the present method is associated with a mutation in EVC gene.

30 c. **Marfan syndrome**

Marfan syndrome is a connective tissue disorder, so affects many

structures, including the skeleton, lungs, eyes, heart and blood vessels. The disease is characterized by unusually long limbs. Marfan syndrome is an autosomal dominant disorder that has been linked to the FBN1 gene on chromosome 15 (Dietz et al., *Nature*, 352(6333):337-9 (1991); and

5 Kainulainen et al., *N. Engl. J. Med.*, 323(14):935-9 (1990)). FBN1 encodes a protein called fibrillin, which is essential for the formation of elastic fibers found in connective tissue. Without the structural support provided by fibrillin, many tissues are weakened, which can have severe consequences, for example, ruptures in the walls of major arteries.

10 In a specific embodiment, the Marfan syndrome to be predicted or diagnosed according to the present method is associated with a mutation in FBN1.

d. Myotonic dystrophy

Myotonic dystrophy is an inherited disorder in which the muscles contract but have decreasing power to relax. With this condition, the muscles also become weak and waste away. Myotonic dystrophy can cause mental deficiency, hair loss and cataracts. Onset of this rare disorder commonly occurs during young adulthood. It can occur at any age and is extremely variable in degree of severity. The myotonic

15 dystrophy gene, found on chromosome 19, codes for a protein kinase that is found in skeletal muscle, where it likely plays a regulatory role (Aslanidis et al., *Nature*, 355(6360):548-51 (1992)). An unusual feature of this illness is that its symptoms usually become more severe with each successive generation. This is because mistakes in the faithful copying of

20 the gene from one generation to the next result in the amplification of a 'AGC triplet repeat', similar to that found in Huntington disease. Unaffected individuals have between 5 and 27 copies of AGC, myotonic

25 dystrophy patients who are minimally affected have at least 50 repeats, while more severely affected patients have an expansion of up to several

30 kilobase pairs.

In a specific embodiment, the myotonic dystrophy to be predicted

or diagnosed according to the present method is associated with a mutation in myotonic dystrophy gene.

##### 5. Nervous system diseases and disorders

Any nervous system diseases and disorders that are associated

- 5 with a mutation(s) in a nucleic acid can be predicted or diagnosed using the present methods. For example, Alzheimer disease (AD), amyotrophic lateral sclerosis (ALS), Angelman syndrome (AS), Charcot-Marie-tooth disease (CMT), epilepsy, tremor, fragile X syndrome, Friedreich's ataxia (FRDA), Huntington disease (HD), Niemann-Pick, Parkinson disease,
- 10 Prader-Willi syndrome (PWS), spinocerebellar atrophy and Williams syndrome can be predicted or diagnosed using the present methods.

###### a. Alzheimer's Disease

Alzheimer' Disease (AD) is the fourth leading cause of death in adults. The incidence of the disease rises steeply with age. Some of the

- 15 most frequently observed symptoms of the disease include a progressive inability to remember facts and events and, later, to recognize friends and family. Certain types of AD run in families: currently, mutations in four genes, situated on chromosomes 1, 14, 19 and 21, are believed to play a role in the disease. The best-characterized of these are PS1 (or AD3) on chromosome 14 and PS2 (or AD4) on chromosome 1 (Levy-Lahad et al., *Science*, 269(5226):973-7 (1995); and Sherrington et al., *Nature*, 375(6534):754-60 (1995)). The formation of lesions made of fragmented brain cells surrounded by amyloid-family proteins are characteristic of the disease. These lesions and their associated proteins
- 20 are closely related to similar structures found in Down's Syndrome. Tangles of filaments largely made up of a protein associated with the cytoskeleton have also been observed in samples taken from Alzheimer brain tissue.

In a specific embodiment, the Alzheimer disease to be predicted or

- 30 diagnosed according to the present method is associated with a mutation in the AD1, AD2, AD3 or AD4 gene.

b. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurological disorder characterized by progressive degeneration of motor neuron cells in the spinal cord and brain, which ultimately results in paralysis and death. The 5 SOD1 gene was identified as being associated with many cases of familial ALS (Rosen et al., *Nature*, 362(6415):59-62 (1993)). The enzyme coded for by SOD1 carries out a very important function in cells: it removes dangerous superoxide radicals by converting them into non-harmful substances. Defects in the action of this enzyme mean that the 10 superoxide radicals attack cells from the inside, causing their death. Several different mutations in this enzyme all result in ALS, making the exact molecular cause of the disease difficult to ascertain.

In a specific embodiment, the amyotrophic lateral sclerosis to be predicted or diagnosed according to the present method is associated 15 with a mutation in SOD1.

c. Angelman syndrome

Angelman syndrome (AS) is an uncommon neurogenetic disorder characterized by mental retardation, abnormal gait, speech impairment, seizures, and an inappropriate happy demeanor which includes frequent 20 laughing, smiling, and excitability. The genetic basis of AS is very complex, but the majority of cases are due to a deletion of segment 15q11-q13 on the maternally derived chromosome 15. When this same region is missing from the paternally derived chromosome, an entirely different disorder, Prader-Willi syndrome, results. This phenomenon - 25 when the expression of genetic material depends on whether it has been inherited from the mother or the father - is termed genomic imprinting. The ubiquitin ligase gene (UBE3A) is found in the AS chromosomal region (Jiang et al., *Am. J. Hum. Genet.*, 65(1):1-6 (1999); Albrecht et al., *Nat. Genet.*, 17(1):75-8 (1997); and Kishino et al., *Nat. Genet.*, 15(1):70-3 30 (1997)). It codes for an enzyme that is a key part of a cellular protein degradation system. AS is thought to occur when mutations in UBE3A

disrupt protein break down during brain development.

In a specific embodiment, the Angelman syndrome to be predicted or diagnosed according to the present method is associated with a mutation in ubiquitin ligase gene (UBE3A).

5           d.     **Charcot-Marie-tooth disease**

Charcot-Marie-tooth disease (CMT) disease is characterized by a slowly progressive degeneration of the muscles in the foot, lower leg, hand and forearm, and a mild loss of sensation in the limbs, fingers and toes. CMT is a genetically heterogeneous disorder, in which mutations in 10 different genes can produce the same clinical symptoms (Lagemann, *ROFO Fortschr Geb Rontgenstr Nuklearmed*, 124(1):69-75 (1976); and Hayasaka et al., *Genomics*, 17(3):755-8 (1993)). In CMT, there are not only different genes but different patterns of inheritance. One of the most common forms of CMT is Type 1A. The gene for Type 1A CMT 15 maps to chromosome 17 and is thought to code for a protein (PMP22) involved in coating peripheral nerves with myelin, a fatty sheath that is important for their conductance. Other types of CMT include Type 1B, autosomal-recessive and X-linked. The same proteins involved in the Type 1A and Type 1B CMT are also involved in a disease called Dejerine- 20 Sottas syndrome (DSS), in which similar clinical symptoms are presented, but they are more severe.

In a specific embodiment, the Charcot-Marie-tooth disease to be predicted or diagnosed according to the present method is associated with a mutation in type 1A or type 1B CMT gene.

25           e.     **Epilepsy**

Epilepsy is characterized by recurring seizures resulting from abnormal cell firing in the brain. There are many forms of epilepsy - most are rare. To date, twelve forms of epilepsy have been demonstrated to possess some genetic basis. For example, LaFora Disease (progressive 30 myoclonic, type 2) is a particularly aggressive epilepsy inherited in an autosomal recessive fashion (Minassian et al., *Nat. Genet.*, 20(2):171-4

(1998)). LaFora Disease is thought to result from a mutation in the EPM2A gene, which is located on chromosome 6. This gene is thought to produce laforin, a protein similar to a group of protein-tyrosine phosphatases that help maintain a balance of sugars in the blood stream.

5 Too much laforin may destroy brain cells, which may then lead to the development of LaFora Disease.

In a specific embodiment, the epilepsy to be predicted or diagnosed according to the present method is associated with a mutation in EPM2A.

f. Tremor

10 Tremor, or uncontrollable shaking, is a common symptom of neurological disorders such as Parkinson's disease, head trauma and stroke. Many people with tremor have what is called idiopathic or essential tremor. In these cases, the tremor itself is the only symptom of the disorder. While essential tremor may involve other parts of the body, 15 the hands and head are most often affected. In more than half of cases, essential tremor is inherited as an autosomal dominant trait, which means that children of an affected individual will have a 50 percent chance of also developing the disorder. In 1997, the ETM1 gene (also called FET1) was mapped to chromosome 3 in a study of Icelandic families, while 20 another gene, called ETM2, was mapped to chromosome 2 in a large American family of Czech descent (Gulcher et al., *Nat. Genet.*, 17(1):84-7 (1997)). That two genes for essential tremor have been found on two different chromosomes demonstrates that mutations in a variety of genes may lead to essential tremor.

25 In a specific embodiment, the tremor to be predicted or diagnosed according to the present method is associated with a mutation in ETM1 or ETM2.

g. Fragile X syndrome

Fragile X syndrome is the most common inherited form of mental 30 retardation currently known. Fragile X syndrome is a defect in the X chromosome and its effects are seen more frequently, and with greater

severity, in males than females. In normal individuals, the FMR1 gene is transmitted stably from parent to child. In Fragile X individuals, there is a mutation in one end of the gene (the 5' untranslated region), that involves amplification of a CGG repeat (Siomi et al., *Cell*, 74(2):291-8 (1993)). Patients with fragile X syndrome have 200 or more copies of the CGG motif. The huge expansion of this repeat means that the FMR1 gene is not expressed, so no FMR1 protein is made. Although the exact function of FMR1 protein in the cell is unclear, it is known that it binds RNA.

10 In a specific embodiment, the fragile X syndrome to be predicted or diagnosed according to the present method is associated with a mutation in FMR1 gene.

h. Friedreich's ataxia

Friedreich's ataxia (FRDA) is a rare inherited disease characterized by the progressive loss of voluntary muscular coordination (ataxia) and heart enlargement. FRDA is an autosomal recessive disease caused by a mutation of a gene called frataxin, which is located on chromosome 9 (Campuzano et al., *Science*, 271(5254):1423-7 (1996); and Babcock et al., *Science*, 276(5319):1709-12 (1997)). This mutation means that there are many extra copies of a DNA segment, the trinucleotide GAA. A normal individual has 8 to 30 copies of this trinucleotide, while FRDA patients have as many as 1000. The larger the number of GAA copies, the earlier the onset of the disease and the quicker the decline of the patient.

25 In a specific embodiment, the Friedreich's ataxia to be predicted or diagnosed according to the present method is associated with a mutation in frataxin.

i. Huntington disease

Huntington disease (HD) is an inherited, degenerative neurological disease that leads to dementia. The HD gene, whose mutation results in Huntington disease, was mapped to chromosome 4 in 1983 and cloned in

1993 (*Cell*, 72(6):971-83 (1993)). The mutation is a characteristic expansion of a nucleotide triplet repeat in the DNA that codes for the protein huntingtin. The number of repeated triplets - CAG (cytosine, adenine, guanine) - increases with the age of the patient. Since people 5 who have those repeats always suffer from Huntington disease, it suggests that the mutation causes a gain-of-function, in which the mRNA or protein takes on a new property or is expressed inappropriately.

In a specific embodiment, the Huntington disease to be predicted or diagnosed according to the present method is associated with a 10 mutation in the HD gene.

j. Niemann-Pick's disease

In 1914, German Pediatrician Albert Niemann described a young child with brain and nervous system impairment. Later, in the 1920's, Luddwick Pick studied tissues after the death of such children and 15 provided evidence of a new disorder, distinct from those storage disorders previously described. Today, there are three separate diseases that carry the name Niemann-Pick: Type A is the acute infantile form, Type B is a less common, chronic, non-neurological form, while Type C is a biochemically and genetically distinct form of the disease. Recently, 20 the major locus responsible for Niemann-Pick type C (NP-C) was cloned from chromosome 18, and found to be similar to proteins that play a role in cholesterol homeostasis (Carstea, *Science*, 277(5323):228-31 (1997); and Loftus, *Science*, 277(5323):232-5 (1997)). Usually, cellular cholesterol is imported into lysosomes - 'bags of enzymes' in the cell - for 25 processing, after which it is released. Cells taken from NP-C patients have been shown to be defective in releasing cholesterol from lysosomes. This leads to an excessive build-up of cholesterol inside lysosomes, causing processing errors. NPC1 was found to have known sterol-sensing regions similar to those in other proteins, which suggests it plays 30 a role in regulating cholesterol traffic.

In a specific embodiment, the Niemann-Pick to be predicted or

diagnosed according to the present method is associated with a mutation in NPC1.

**k. Parkinson disease**

Parkinson disease is a neurodegenerative disease that manifests as

5 a tremor, muscular stiffness and difficulty with balance and walking. A classic pathological feature of the disease is the presence of an inclusion body, called the Lewy body, in many regions of the brain. A candidate gene for some cases of Parkinson disease was mapped to chromosome 4 (Polymeropoulos et al., *Science*, 276(5321):2045-7 (1997)). Mutations  
10 in this gene have now been linked to several Parkinson disease families. The product of this gene, a protein called alpha-synuclein, is a familiar culprit: a fragment of it is a known constituent of Alzheimer disease plaques.

In a specific embodiment, the Parkinson disease to be predicted or  
15 diagnosed according to the present method is associated with a mutation in  $\alpha$ -synuclein.

**I. Spinocerebellar atrophy**

Persons with spinocerebellar atrophy, of which there are several types, experience a degeneration of the spinal cord and the cerebellum,  
20 the small fissured mass at the base of the brain, behind the brain stem. The cerebellum is concerned with coordination of movements, so atrophy or "wasting away" of this critical control center results in a loss of muscle coordination. Atrophy in the spine can bring spasticity. The basic defect in all types of spinocerebellar atrophy is a an expansion of a  
25 CAG triplet repeat. In this way, it is similar to fragile-X syndrome, Huntington disease and myotonic dystrophy, all of which exhibit a triplet repeat expansion of a gene. In the case of spinocerebellar atrophy I, the gene is SCA1, found on chromosome 6 (Banfi et al., *Nat. Genet.*, 7(4):513-20 (1994)). The protein product of the gene - called ataxin-1 -  
30 varies in size, depending on the size of the CAG triplet repeat.

In a specific embodiment, the Prader-Willi syndrome to be predicted

or diagnosed according to the present method is associated with a mutation in the small ribonucleoprotein N (SNRPN).

m. Williams syndrome

Williams syndrome is a rare congenital disorder characterized by

- 5 physical and development problems. Common features include characteristic "elfin-like" facial features, heart and blood vessel problems, irritability during infancy, dental and kidney abnormalities, hyperacusis (sensitive hearing) and musculoskeletal problems. In Williams syndrome individuals, the gene for elastin and an enzyme called LIM kinase are
- 10 deleted (Frangiskakis et al., *Cell*, 86(1):59-69 (1996); and Lenhoff et al., *Sci. Am.*, 277(6):68-73 (1997)). Both genes map to the same small area on chromosome 7. In normal cells, elastin is a key component of connective tissue, conferring its elastic properties. Mutation or deletion of elastin lead to the vascular disease observed in Williams syndrome. On
- 15 the other hand, LIM kinase is strongly expressed in the brain, and deletion of LIM kinase is thought to account for the impaired visuospatial constructive cognition in Williams syndrome. Williams syndrome is a contiguous disease, meaning that the deletion of this section of chromosome 7 may involve several more genes. Further study will be
- 20 required to round up all the genes deleted in this disease.

In a specific embodiment, the Williams syndrome to be predicted or diagnosed according to the present method is associated with a mutation in elastin and LIM kinase.

6. Signal disease or disorder

- 25 Any signal diseases or disorders that are associated with a mutation(s) in a nucleic acid can be predicted or diagnosed using the present methods. For example, ataxia telangiectasia (A-T), male pattern baldness, acne, hirsutism, Cockayne syndrome, glaucoma, mammals with abnormal secondary sexual characteristics, tuberous sclerosis,
- 30 Waardenburg syndrome (WS) and Werner syndrome (WRN) can be predicted or diagnosed using the present methods.

a. Ataxia telangiectasia

The first signs of ataxia telangiectasia (A-T) usually appear in the second year of life as a lack of balance and slurred speech. It is a progressive, degenerative disease characterized by cerebellar degeneration, immunodeficiency, radiosensitivity (sensitivity to radiant energy, such as x-ray) and a predisposition to cancer. The gene responsible for A-T was mapped to chromosome 11. The subsequent identification of the gene proved difficult: it was seven more years until the human ATM gene was cloned (Savitsky, *Science*, 268(5218):1749-53 (1995); and Barlow *Cell*, 86(1):159-71 (1996)). The diverse symptoms seen in A-T reflect the main role of ATM, which is to induce several cellular responses to DNA damage. When the ATM gene is mutated, these signaling networks are impaired and so the cell does not respond correctly to minimize the damage.

In a specific embodiment, the ataxia telangiectasia to be predicted or diagnosed according to the present method is associated with a mutation in ATM.

b. Male pattern baldness, acne or hirsutism

Five- $\alpha$  reductase is an enzyme that was first discovered in the male prostate. Here, it catalyzes the conversion of testosterone to dihydrotestosterone, which in turn binds to the androgen receptor and initiates development of the external genitalia and prostate. The gene for 5-alpha reductase has been mapped to chromosome 5 (Andersson and Russell, *Proc. Natl. Acad. Sci.*, 87(10):3640-4 (1990); and Jenkins *Genomics*, 11(4):1102-12 (1991)). More recently, 5-alpha reductase was found in human scalp and elsewhere in the skin, where it carries out the same reaction as in the prostate. It is thought that disturbances in 5-alpha reductase activity in skin cells might contribute to male pattern baldness, acne or hirsutism.

In a specific embodiment, the male pattern baldness, acne or

hirsutism to be predicted or diagnosed according to the present method is associated with a mutation in 5- $\alpha$  reductase.

c. Cockayne syndrome

Cockayne syndrome is a rare inherited disorder in which people are

5 sensitive to sunlight, have short stature and have the appearance of premature aging. In the classical form of Cockayne syndrome (Type I), the symptoms are progressive and typically become apparent after the age of one year. An early onset or congenital form of Cockayne syndrome (Type II) is apparent at birth. Interestingly, unlike other DNA repair diseases, Cockayne syndrome is not linked to cancer. After exposure to UV radiation (found in sunlight), people with Cockayne syndrome can no longer perform a certain type of DNA repair, known as 'transcription-coupled repair'. This type of DNA repair occurs 'on the fly', right as the DNA that codes for proteins is being replicated. Two genes

10 defective in Cockayne syndrome, CSA and CSB, have been identified so far. The CSA gene is found on chromosome 5. Both genes code for proteins that interacts with components of the transcriptional machinery and with DNA repair proteins (van Gool, *EMBO J.*, 16(14):4155-62 (1997)).

15

20 In a specific embodiment, the Cockayne syndrome to be predicted or diagnosed according to the present method is associated with a mutation in CSA or CSB.

d. Glaucoma

Glaucoma is a term used for a group of diseases that can lead to

25 damage to the eye's optic nerve and result in blindness. The most common form of the disease is open-angle glaucoma, which affects about three million Americans, half of whom don't know they have it. Glaucoma has no symptoms at first but over the years can steal its victims' sight, with side vision being effected first. It is estimated that

30 nearly 100,000 individuals in the US suffer from glaucoma due to a mutation in the GLC1A gene, found on chromosome 1 (Stone, *Science*,

275(5300):668-70 (1997)). There has been some speculation as to the role of the gene product in the eye. As it is found in the structures of the eye involved in pressure regulation, it may cause increased pressure in the eye by obstructing the aqueous outflow.

5 In a specific embodiment, the glaucoma to be predicted or diagnosed according to the present method is associated with a mutation in GLC1A.

e. **Abnormal secondary sexual characteristics**

Usually, a woman has two X chromosomes (XX) and a man one X and one Y (XY). Male and female characteristics sometimes can be found in one individual, and it is possible to have XY women and XX men. Analysis of such individuals has revealed some of the molecules involved in sex determination, including one called SRY, which is important for testis formation. SRY (which stands for sex-determining region Y gene) is found on the Y chromosome (*Berta, Nature, 348(6300):448-50 (1990); and Goodfellow and Lovell-Badge, Annu. Rev. Genet., 27:71-92 (1993))*. In the cell, it binds to DNA and in doing so distorts it dramatically out of shape. This alters the properties of the DNA and likely alters the expression of a number of genes, leading to testis formation. Therefore XX men who lack a Y chromosome also lack SRY and frequently do not develop secondary sexual characteristics in the usual way.

In a specific embodiment, the abnormal secondary sexual characteristics to be predicted or diagnosed according to the present method is associated with a mutation in sex-determining region Y gene (SRY).

f. **Tuberous sclerosis**

Tuberous sclerosis is an hereditary disorder characterized by benign, tumor-like nodules of the brain and/or retinas, skin lesions, seizures and/or mental retardation. Patients may experience a few or all of the symptoms with varying degrees of severity. Two loci for tuberous

sclerosis have been found: TSC1 on chromosome 9, and TSC2 on chromosome 16 (*Cell*, 75(7):1305-15 (1993)). It took four years to pin down a specific gene from the TSC1 region of chromosome 9: in 1997, a promising candidate was found. Called hamartin by the discoverers, it is

5 similar to a yeast protein of unknown function, and appears to act as a tumor suppressor: without TSC1, growth of cells proceeds in an unregulated fashion, resulting in tumor formation (van Slegtenhorst, *Science*, 277(5327):805-8 (1997)). TSC2 codes for a protein called tuberin, which, through database searches, was found to have a region of

10 homology to a protein found in pathways that regulate the cell (GAP3, a GTPase-activation protein).

In a specific embodiment, the tuberous sclerosis to be predicted or diagnosed according to the present method is associated with a mutation in TSC1 or TSC2.

15                   g.     Waardenburg syndrome

The main characteristics of Waardenburg syndrome (WS) include: a wide bridge of the nose; pigmentary disturbances such as two different colored eyes, white forelock and eyelashes and premature graying of the hair; and some degree of cochlear deafness. The several types of WS are

20 inherited in dominant fashion, so researchers typically see families with several generations who have inherited one or more of the features. Type I of the disorder is characterized by displacement of the fold of the eyelid, while Type II does not include this feature, but instead has a higher frequency of deafness. The discovery of the human gene that

25 causes Type I WS came about after scientists speculated that the gene that causes 'splotch mice' (mice with a splotchy coat coloring) might be the same gene that causes WS in humans. They located the human gene to chromosome 2 and found it was the same as mouse Pax3 (Tassabehji et al., *Nature*, 355(6361):635-6 (1992)).

30                   In a specific embodiment, the Waardenburg syndrome to be predicted or diagnosed according to the present method is associated

with a mutation in human homolog of mouse Pax3.

h. Werner syndrome

Werner syndrome is a premature aging disease that begins in adolescence or early adulthood and results in the appearance of old age

5 by 30-40 years of age. Its physical characteristics may include short stature (common from childhood on) and other features usually developing during adulthood: wrinkled skin, baldness, cataracts, muscular atrophy and a tendency to diabetes mellitus, among others. The disorder is inherited and transmitted as an autosomal recessive trait. Cells from

10 WS patients have a shorter lifespan in culture than do normal cells. The gene for Werner disease (WRN) was mapped to chromosome 8 and cloned: by comparing its sequence to existing sequences in GenBank, it is a predicted helicase belonging to the RecQ family (Gray et al., *Nat. Genet.*, 17(1):100-3 (1997); and Sinclair et al., *Science*,

15 277(5330):1313-6 (1997)).

In a specific embodiment, the Werner syndrome to be predicted or diagnosed according to the present method is associated with a mutation in WRN gene.

7. Transporter diseases and disorders

20 Any transporter diseases and disorders that are associated with a mutation(s) in a nucleic acid can be predicted or diagnosed using the present methods. For example, cystic fibrosis (CF), diastrophic dysplasia (DTD), long-QT syndrome (LQTS), Menkes' syndrome, pendred syndrome, adult polycystic kidney disease (APKD), Wilson's disease and

25 Zellweger syndrome can be predicted or diagnosed using the present methods.

a. Cystic fibrosis

Cystic fibrosis (CF) is the most common fatal genetic disease in the US today. It causes the body to produce a thick, sticky mucus that clogs

30 the lungs, leading to infection, and blocks the pancreas, stopping digestive enzymes from reaching the intestines where they are required to

digest food. CF is caused by a defective gene, which codes for a sodium and chloride (salt) transporter found on the surface of the epithelial cells that line the lungs and other organs (Riordan et al., *Science*, 245(4922):1066-73 (1989)). Several hundred mutations have been 5 found in this gene, all of which result in defective transport of sodium and chloride by epithelial cells. The severity of the disease symptoms of CF is directly related to the characteristic effects of the particular mutation(s) that have been inherited by the sufferer.

In a specific embodiment, the cystic fibrosis to be predicted or 10 diagnosed according to the present method is associated with a mutation in the CF gene.

b. **Diastrophic dysplasia**

Diastrophic dysplasia (DTD) is a rare growth disorder in which patients are usually short, have club feet and have malformed hands and 15 joints. Although found in all populations, it is particularly prevalent in Finland. The gene whose mutation results in DTD maps to chromosome 5 and encodes a novel sulfate transporter (Hastbacka et al., *Genomics*, 11(4):968-73 (1991); and Hastbacka et al., *Cell*, 78(6):1073-87 (1994)). This ties in with the observation of unusual concentrations of sulfate in 20 various tissues of DTD patients. Sulfate is important for skeletal joints because cartilage - the shock-absorber of joints - requires sulfur during its manufacture. Adding sulfur increases the negative charge within cartilage, which contributes to its shock-absorbing properties.

In a specific embodiment, the diastrophic dysplasia to be predicted 25 or diagnosed according to the present method is associated with a mutation in the DTD gene.

c. **Long-QT syndrome**

Long-QT syndrome (LQTS) results from structural abnormalities in the potassium channels of the heart, which predispose affected persons 30 to an accelerated heart rhythm (arrhythmia). This can lead to sudden loss of consciousness and may cause sudden cardiac death in teenagers and

young adults who are faced with stressors ranging from exercise to loud sounds. LQTS is usually inherited as an autosomal dominant trait (Wang et al., *Nat. Genet.*, 12(1):17-23 (1996); and Barhanin et al., *Nature*, 384(6604):78-80 (1996)). In the case of LQT1, which has been mapped

5 to chromosome 11, mutations lead to serious structural defects in the person's cardiac potassium channels that do not allow proper transmission of the electrical impulses throughout the heart. There also appear to be other genes, tentatively located on chromosomes 3, 6 and 11 whose mutated products may contribute to, or cause, LQT syndrome.

10 In a specific embodiment, the long-QT syndrome to be predicted or diagnosed according to the present method is associated with a mutation in LQT1.

d. Menkes' syndrome

Menkes' syndrome is an inborn error of metabolism that markedly decreases the cells' ability to absorb copper. The disorder causes severe cerebral degeneration and arterial changes, resulting in death in infancy. The disease can often be diagnosed by looking at a victim's hair, which appears to be whitish and kinked when viewed under a microscope. Menkes' disease is transmitted as an X-linked recessive trait. Sufferers can not transport copper, which is needed by enzymes involved in making bone, nerve and other structures (Chelly et al., *Nat. Genet.*, 3(1):14-9 (1993)). A number of other diseases, including type IX Ehlers-Danlos syndrome, may be the result of allelic mutations (*i.e.*, mutations in the same gene, but having slightly different symptoms) and it is hoped that research into these diseases may prove useful in fighting Menkes' disease.

In a specific embodiment, the Menkes' syndrome to be predicted or diagnosed according to the present method is associated with a mutation in the copper transporter.

30 e. Pendred syndrome

Pendred syndrome is an inherited disorder that accounts for as

much as 10% of hereditary deafness. Patients usually also suffer from thyroid goiter. In December of 1997, scientists at NIH's National Human Genome Research Institute used the physical map of human chromosome 7 to help identify an altered gene thought to cause pendred syndrome

5 (Everett et al., *Nat. Genet.*, 17(4):411-22 (1997)). The normal gene makes a protein, called pendrin, that is found at significant levels only in the thyroid and is closely related to a number of sulfate transporters. When the gene for this protein is mutated, the person carrying it will exhibit the symptoms of Pendred syndrome.

10 In a specific embodiment, the pendred syndrome to be predicted or diagnosed according to the present method is associated with a mutation in pendrin.

f. **Adult polycystic kidney disease**

Adult polycystic kidney disease (APKD) is characterized by large

15 cysts in one or both kidneys and a gradual loss of normal kidney tissue. The role of the kidneys in the body is to filter the blood, excreting the end-products of metabolism in the form of urine and regulating the concentrations of hydrogen, sodium, potassium, phosphate and other ions in the extracellular fluid. Patients with APKD can die from renal

20 failure, or from the consequences of hypertension (high arterial blood pressure). In 1994 the European Polycystic Kidney Disease Consortium isolated a gene from chromosome 16 that was disrupted in a family with APCD (*Cell*, 77(6):881-94 (1994) (Published errata appear in *Cell* 1994 Aug 26;78(4):following 724 and 1995 Jun 30;81(7):following 1170);

25 and *Cell*, 81(2):289-98 (1995)). The protein encoded by the PKD1 gene is an integral membrane protein involved in cell-cell interactions and cell-matrix interactions. The role of PKD1 in the normal cell may be linked to microtubule-mediated functions, such as the placement of Na(+), K(+)-ATPase ion pumps in the membrane. Programmed cell death, or

30 apoptosis, may also be invoked in APKD.

In a specific embodiment, the adult polycystic kidney disease to be

predicted or diagnosed according to the present method is associated with a mutation in PKD1.

g. **Wilson's disease**

Wilson's disease is a rare autosomal recessive disorder of copper transport, resulting in copper accumulation and toxicity to the liver and brain. Liver disease is the most common symptom in children; neurological disease is most common in young adults. The cornea of the eye can also be affected: the 'Kayser-Fleischer ring' is a deep copper-colored ring at the periphery of the cornea, and is thought to represent copper deposits. The gene for Wilson's disease (ATP7B) was mapped to chromosome 13. The sequence of the gene was found to be similar to sections of the gene defective in Menkes disease, another disease caused by defects in copper transport. The similar sequences code for copper-binding regions, which are part of a transmembrane pump called a P-type ATPase that is very similar to the Menkes disease protein (Bull et al., Nat. Genet., 5(4):327-37 (1993) (Published erratum appears in Nat Genet 1994 Feb;6(2):214).

In a specific embodiment, the Wilson's disease to be predicted or diagnosed according to the present method is associated with a mutation in ATP7B.

h. **Zellweger syndrome**

Zellweger syndrome is a rare hereditary disorder affecting infants, and usually results in death. Unusual problems in prenatal development, an enlarged liver, high levels of iron and copper in the blood, and vision disturbances are among the major manifestations of Zellweger syndrome. The PXR1 gene has been mapped to chromosome 12; mutations in this gene cause Zellweger syndrome. The PXR1 gene product is a receptor found on the surface of peroxisomes - microbodies found in animal cells, especially liver, kidney and brain cells (Dodd et al., Nat. Genet., 9(2):115-25 (1995); and Marynen et al., *Genomics*, 30(2):366-8 (1995)). The PXR1 receptor is vital for the import of these enzymes into the

peroxisomes: without it functioning properly, the peroxisomes can not use the enzymes to carry out their important functions, such as cellular lipid metabolism and metabolic oxidations.

In a specific embodiment, the Zellweger syndrome to be predicted  
5 or diagnosed according to the present method is associated with a mutation in PXR1.

#### 8. Infections

Any infections by pathological agents can be predicted or diagnosed using the present methods. For example, infections by  
10 viruses, eubacteria, archaebacteria and eukaryotic pathogens can be predicted or diagnosed using the present methods.

In a specific embodiment, the viral infection to be predicted or diagnosed according to the present method is caused by a Delta virus, a dsDNA virus, a retrovirus, a satellite virus, a ssDNA virus, a ssRNA  
15 negative-strand virus, ssRNA positive-strand virus (no DNA stage) or a bacteriophage.

In another specific embodiment, the eubacteria infection to be predicted or diagnosed according to the present method is caused by a green bacteria, a flavobacteria, a spirochete, a purple bacteria, a gram-  
20 positive bacteria, a gram-negative bacteria, a cyanobacteria, a deinococci or a thermotogale.

In still another specific embodiment, the archaebacteria infection to be predicted or diagnosed according to the present method is caused by an extreme halophile, a methanogen or an extreme thermophile.

25 In yet another specific embodiment, the infection to be predicted or diagnosed according to the present method is caused by an eukaryotic pathogen such as a fungi, a ciliate, a cellular slime mold, a flagellate or a microsporidia.

#### D. METHODS FOR DETECTING POLYMORPHISMS

30 Provided herein is a method for detecting polymorphism in a locus, which method comprises: a) hybridizing a target strand of a nucleic acid

comprising a locus to be tested with a complementary reference strand of a nucleic acid comprising a known allele of the locus, whereby the allelic identity between the target and the reference strands results in the formation of a nucleic acid duplex without an abnormal base-pairing and

5 the allelic difference between the target and the reference strands results in the formation of a nucleic acid duplex with an abnormal base-pairing;

b) contacting the nucleic acid duplex formed in step a) with a mutant DNA repair enzyme or complex thereof, wherein the mutant DNA repair enzyme or complex thereof has binding affinity for the abnormal base-

10 pairing in the duplex but has attenuated catalytic activity; and c)

detecting binding between the nucleic acid duplex and the mutant DNA repair enzyme or complex thereof, whereby the polymorphism in the locus is assessed.

In a specific embodiment, the polymorphism to be detected is a

15 variable nucleotide type polymorphism ("VNTR").

In another specific embodiment, the polymorphism to be detected is a single nucleotide polymorphism (SNP). Preferably, a polymorphism in a genome, e.g., a viral, bacterial, eukaryotic, mammalian or human genome, is detected by the present methods. More preferably, the

20 human genome SNPs listed in the following Table 2 can be detected by the present methods (see e.g., <http://www.ncbi.nlm.gov/SNP>).

**Table 2. Examples of human genome polymorphisms**

25	<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL</u>
				<u>SNP ID</u>
30	1	0.00 cR from top of Chr1 linka	1946	WIAF   WIAF-3885
		0.00 cR from top of Chr1 linka	2870	WIAF   WIAF-768
		0.60 cR from top of Chr1 linka	1196	WIAF   WIAF-2083
		6.20 cR from top of Chr1 linka	1861	WIAF   WIAF-3800
		7.8 cR from top of Chr1 linkag	2383	WIAF   WIAF-2674
		12.1 cR from top of Chr1 linka	3083	WIAF   WIAF-984
35	1	16.40 cR from top of Chr1 link	1921	WIAF   WIAF-3860
		21.2 cR from top of Chr1 linka	3061	WIAF   WIAF-962
		23.3 cR from top of Chr1 linka	2762	WIAF   WIAF-501
		27.10 cR from top of Chr1 link	1421	WIAF   WIAF-3349
		33.30 cR from top of Chr1 link	2934	WIAF   WIAF-833

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
5	1 34.50 cR from top of Chr1 link	3318	WIAF   WIAF-1771
	1 50.0 cR from top of Chr1 link	2566	WIAF   WIAF-195
	1 50.40 cR from top of Chr1 link	1954	WIAF   WIAF-3893
	1 51.20 cR from top of Chr1 link	3248	WIAF   WIAF-1663
5	1 54.9 cR from top of Chr1 link	3124	WIAF   WIAF-1025
	1 55.5 cR from top of Chr1 link	2576	WIAF   WIAF-206
	1 55.80 cR from top of Chr1 link	1130	WIAF   WIAF-1577
	1 55.80 cR from top of Chr1 link	1131	WIAF   WIAF-1578
10	1 55.80 cR from top of Chr1 link	2951	WIAF   WIAF-850
	1 55.90 cR from top of Chr1 link	670	WIAF   WIAF-1348
	1 57.00 cR from top of Chr1 link	3255	WIAF   WIAF-1677
	1 59.80 cR from top of Chr1 link	2526	WIAF   WIAF-135
	1 60 cM	4319	UWGC   138
	1 60.70 cR from top of Chr1 link	1498	WIAF   WIAF-3437
15	1 62.8 cR from top of Chr1 link	2079	WIAF   WIAF-28
	1 68.5 cR from top of Chr1 link	3138	WIAF   WIAF-1039
	1 69.00 cR from top of Chr1 link	3043	WIAF   WIAF-944
	1 71.30 cR from top of Chr1 link	3188	WIAF   WIAF-1504
20	1 75.30 cR from top of Chr1 link	3479	WIAF   WIAF-1934
	1 75.90 cR from top of Chr1 link	1886	WIAF   WIAF-3825
	1 77.20 cR from top of Chr1 link	1275	WIAF   WIAF-2162
	1 77.90 cR from top of Chr1 link	677	WIAF   WIAF-1443
	1 78.30 cR from top of Chr1 link	2876	WIAF   WIAF-774
	1 78.60 cR from top of Chr1 link	1179	WIAF   WIAF-1708
25	1 84.30 cR from top of Chr1 link	1756	WIAF   WIAF-3695
	1 91.5 cR from top of Chr1 link	743	WIAF   WIAF-1191
	1 92.60 cR from top of Chr1 link	1388	WIAF   WIAF-3293
	1 97.8 cR from top of Chr1 link	2273	WIAF   WIAF-734
	1 103.20 cR from top of Chr1 lin	1622	WIAF   WIAF-3561
30	1 103.20 cR from top of Chr1 lin	1626	WIAF   WIAF-3565
	1 106.90 cR from top of Chr1 lin	1577	WIAF   WIAF-3516
	1 113.3 cR from top of Chr1 link	2554	WIAF   WIAF-178
	1 117.4 cR from top of Chr1 link	975	WIAF   WIAF-1388
	1 118.70 cR from top of Chr1 lin	2527	WIAF   WIAF-136
35	1 118.70 cR from top of Chr1 lin	1952	WIAF   WIAF-3891
	1 119.10 cR from top of Chr1 lin	2032	WIAF   WIAF-1590
	1 120.30 cR from top of Chr1 lin	3229	WIAF   WIAF-1630
	1 129.30 cR from top of Chr1 lin	1873	WIAF   WIAF-3812
	1 129.30 cR from top of Chr1 lin	1876	WIAF   WIAF-3815
40	1 129.30 cR from top of Chr1 lin	1877	WIAF   WIAF-3816
	1 129.40 cR from top of Chr1 lin	1157	WIAF   WIAF-1642
	1 141.60 cR from top of Chr1 lin	1110	WIAF   WIAF-1543
	1 142.9 cR from top of Chr1 link	2123	WIAF   WIAF-298
	1 142.9 cR from top of Chr1 link	2124	WIAF   WIAF-299
45	1 146.90 cR from top of Chr1 lin	1859	WIAF   WIAF-3798
	1 147.90 cR from top of Chr1 lin	3552	WIAF   WIAF-2007
	1 147.90 cR from top of Chr1 lin	1693	WIAF   WIAF-3632
	1 148.10 cR from top of Chr1 lin	3053	WIAF   WIAF-954
	1 148.30 cR from top of Chr1 lin	1186	WIAF   WIAF-2073
50	1 154.00 cR from top of Chr1 lin	1263	WIAF   WIAF-2150

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>		<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	1 156.10 cR from top of Chr1 lin	1266	WIAF	WIAF-2153
	1 156.10 cR from top of Chr1 lin	1267	WIAF	WIAF-2154
	1 160.30 cR from top of Chr1 lin	1945	WIAF	WIAF-3884
	1 160.50 cR from top of Chr1 lin	1369	WIAF	WIAF-3272
5	1 161.9 cR from top of Chr1 link	1077	WIAF	WIAF-2040
	1 162.40 cR from top of Chr1 lin	1140	WIAF	WIAF-1603
	1 162.90 cR from top of Chr1 lin	3038	WIAF	WIAF-939
	1 164.10 cR from top of Chr1 lin	3574	WIAF	WIAF-2029
	1 164.10 cR from top of Chr1 lin	3575	WIAF	WIAF-2030
10	1 164.10 cR from top of Chr1 lin	1357	WIAF	WIAF-3260
	1 164.60 cR from top of Chr1 lin	1566	WIAF	WIAF-3505
	1 166.90 cR from top of Chr1 lin	3466	WIAF	WIAF-1921
	1 168.60 cR from top of Chr1 lin	1295	WIAF	WIAF-2182
	1 168.60 cR from top of Chr1 lin	1296	WIAF	WIAF-2183
15	1 169.40 cR from top of Chr1 lin	1930	WIAF	WIAF-3869
	1 170.30 cR from top of Chr1 lin	1641	WIAF	WIAF-3580
	1 170.30 cR from top of Chr1 lin	1644	WIAF	WIAF-3583
	1 171.5 cR from top of Chr1 link	2853	WIAF	WIAF-740
	1 174.50 cR from top of Chr1 lin	1751	WIAF	WIAF-3690
20	1 182.20 cR from top of Chr1 lin	1731	WIAF	WIAF-3670
	1 182.30 cR from top of Chr1 lin	2034	WIAF	WIAF-1595
	1 182.80 cR from top of Chr1 lin	3437	WIAF	WIAF-1892
	1 183.30 cR from top of Chr1 lin	1982	WIAF	WIAF-3921
	1 183.8 cR from top of Chr1 link	3593	WIAF	WIAF-2069
25	1 187.20 cR from top of Chr1 lin	2450	WIAF	WIAF-38
	1 188.30 cR from top of Chr1 lin	2868	WIAF	WIAF-766
	1 191.30 cR from top of Chr1 lin	1521	WIAF	WIAF-3460
	1 192.40 cR from top of Chr1 lin	1458	WIAF	WIAF-3391
	1 192.50 cR from top of Chr1 lin	1445	WIAF	WIAF-3375
30	1 198.30 cR from top of Chr1 lin	1360	WIAF	WIAF-3263
	1 198.7 cR from top of Chr1 link	2224	WIAF	WIAF-653
	1 199.30 cR from top of Chr1 lin	3393	WIAF	WIAF-1848
	1 200.80 cR from top of Chr1 lin	1224	WIAF	WIAF-2111
	1 201.00 cR from top of Chr1 lin	1245	WIAF	WIAF-2132
35	1 204.40 cR from top of Chr1 lin	1235	WIAF	WIAF-2122
	1 209.90 cR from top of Chr1 lin	2911	WIAF	WIAF-809
	1 213.0 cR from top of Chr1 link	983	WIAF	WIAF-1409
	1 216.50 cR from top of Chr1 lin	1477	WIAF	WIAF-3415
	1 217.60 cR from top of Chr1 lin	1995	WIAF	WIAF-3934
40	1 218.0 cR from top of Chr1 link	2947	WIAF	WIAF-846
	1 221.70 cR from top of Chr1 lin	1191	WIAF	WIAF-2078
	1 224.60 cR from top of Chr1 lin	2006	WIAF	WIAF-1470
	1 224.70 cR from top of Chr1 lin	1823	WIAF	WIAF-3762
	1 228.50 cR from top of Chr1 lin	1585	WIAF	WIAF-3524
45	1 228.50 cR from top of Chr1 lin	1590	WIAF	WIAF-3529
	1 231.2 cR from top of Chr1 link	3142	WIAF	WIAF-1043
	1 231.2 cR from top of Chr1 link	3544	WIAF	WIAF-1999
	1 232.00 cR from top of Chr1 lin	3326	WIAF	WIAF-1779
	1 232.40 cR from top of Chr1 lin	3518	WIAF	WIAF-1973
50	1 235.30 cR from top of Chr1 lin	1262	WIAF	WIAF-2149

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
1	236.3 cR from top of Chr1 link	2877	WIAF   WIAF-775
1	246.20 cR from top of Chr1 lin	1491	WIAF   WIAF-3430
1	247.30 cR from top of Chr1 lin	1747	WIAF   WIAF-3686
1	247.4 cR from top of Chr1 link	2654	WIAF   WIAF-328
5	1 247.4 cR from top of Chr1 link	2655	WIAF   WIAF-329
1	248.00 cR from top of Chr1 lin	1211	WIAF   WIAF-2098
1	249.00 cR from top of Chr1 lin	1508	WIAF   WIAF-3447
1	249.80 cR from top of Chr1 lin	3112	WIAF   WIAF-1013
1	249.80 cR from top of Chr1 lin	3113	WIAF   WIAF-1014
10	1 250.10 cR from top of Chr1 lin	704	WIAF   WIAF-1344
1	250.10 cR from top of Chr1 lin	1113	WIAF   WIAF-1548
1	251.00 cR from top of Chr1 lin	3559	WIAF   WIAF-2014
1	253.2 cR from top of Chr1 link	3399	WIAF   WIAF-1854
1	254.7 cR from top of Chr1 link	2643	WIAF   WIAF-312
15	1 254.7 cR from top of Chr1 link	2966	WIAF   WIAF-866
1	256.10 cR from top of Chr1 lin	1102	WIAF   WIAF-1521
1	258.70 cR from top of Chr1 lin	1185	WIAF   WIAF-2072
1	263.8 cR from top of Chr1 link	3295	WIAF   WIAF-1748
1	273.20 cR from top of Chr1 lin	1236	WIAF   WIAF-2123
20	1 281.00 cR from top of Chr1 lin	3224	WIAF   WIAF-1616
1	282.70 cR from top of Chr1 lin	3348	WIAF   WIAF-1801
1	284.3 cR from top of Chr1 link	3388	WIAF   WIAF-1842
1	286.6 cR from top of Chr1 link	2075	WIAF   WIAF-11
1	292.70 cR from top of Chr1 lin	1630	WIAF   WIAF-3569
25	1 369.7 cR from top of Chr1 link	2941	WIAF   WIAF-840
1	454.8 cR from top of Chr1 link	2910	WIAF   WIAF-808
1	458.7 cR from top of Chr1 link	2462	WIAF   WIAF-53
1	477.3 cR from top of Chr1 link	3922	WIAF   WIAF-4010
1	557.1 cR from top of Chr1 link	2381	WIAF   WIAF-2667
30	1 573.5 cR from top of Chr1 link	2741	WIAF   WIAF-455
1	629.9 cR from top of Chr1 link	3592	WIAF   WIAF-2068
1	639.0 cR from top of Chr1 link	772	WIAF   WIAF-1403
1	646.6 cR from top of Chr1 link	1078	WIAF   WIAF-2044
1	674.3 cR from top of Chr1 link	3856	WIAF   WIAF-2644
35	1 675.4 cR from top of Chr1 link	2482	WIAF   WIAF-79
1	676.5 cR from top of Chr1 link	2555	WIAF   WIAF-179
1	676.5 cR from top of Chr1 link	3501	WIAF   WIAF-1956
1	680.0 cR from top of Chr1 link	4585	HU-CHINA   1-1328
1	80.0 cR from top of Chr1 link	4558	HU-CHINA   1-1328-2
40	1 680.0 cR from top of Chr1 link	4559	HU-CHINA   1-1328-3
1	680.0 cR from top of Chr1 link	759	WIAF   WIAF-1328
1	684.2 cR from top of Chr1 link	3067	WIAF   WIAF-968
1	684.2 cR from top of Chr1 link	3068	WIAF   WIAF-969
1	692.5 cR from top of Chr1 link	2715	WIAF   WIAF-413
45	1 695.0 cR from top of Chr1 link	2959	WIAF   WIAF-858
1	702.0 cR from top of Chr1 link	2623	WIAF   WIAF-282
1	732.4 cR from top of Chr1 link	2223	WIAF   WIAF-652
1	749.9 cR from top of Chr1 link	2250	WIAF   WIAF-696
50	1 759.2 cR from top of Chr1 link	2586	WIAF   WIAF-221
50	1 769.0 cR from top of Chr1 link	2810	WIAF   WIAF-590

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
1	769.1 cR from top of Chr1 link	769	WIAF   WIAF-1389
1	770.3 cR from top of Chr1 link	3448	WIAF   WIAF-1903
1	781.7 cR from top of Chr1 link	3004	WIAF   WIAF-904
1	783.2 cR from top of Chr1 link	2086	WIAF   WIAF-95
5	817.5 cR from top of Chr1 link	976	WIAF   WIAF-1390
1	819.6 cR from top of Chr1 link	3395	WIAF   WIAF-1850
1	820.1 cR from top of Chr1 link	895	WIAF   WIAF-1143
1	820.1 cR from top of Chr1 link	1006	WIAF   WIAF-4029
10	823.3 cR from top of Chr1 link	2088	WIAF   WIAF-102
1	823.3 cR from top of Chr1 link	2089	WIAF   WIAF-103
1	838.6 cR from top of Chr1 link	2232	WIAF   WIAF-665
1	873.2 cR from top of Chr1 link	2618	WIAF   WIAF-269
1	873.2 cR from top of Chr1 link	2619	WIAF   WIAF-270
15	875.1 cR from top of Chr1 link	3850	WIAF   WIAF-2636
1	883.1 cR from top of Chr1 link	2540	WIAF   WIAF-154
1	884.8 cR from top of Chr1 link	2867	WIAF   WIAF-765
1	889.8 cR from top of Chr1 link	3051	WIAF   WIAF-952
1	890.2 cR from top of Chr1 link	3116	WIAF   WIAF-1017
1	890.3 cR from top of Chr1 link	3841	WIAF   WIAF-2617
20	910.7 cR from top of Chr1 link	2983	WIAF   WIAF-883
1	917.7 cR from top of Chr1 link	3042	WIAF   WIAF-943
1	943.9 cR from top of Chr1 link	2525	WIAF   WIAF-134
1	947.6 cR from top of Chr1 link	2885	WIAF   WIAF-783
1	951.7 cR from top of Chr1 link	2935	WIAF   WIAF-834
25	959.3 cR from top of Chr1 link	3283	WIAF   WIAF-1736
1	959.3 cR from top of Chr1 link	2424	WIAF   WIAF-4
1	961.2 cR from top of Chr1 link	2570	WIAF   WIAF-200
1	961.3 cR from top of Chr1 link	2782	WIAF   WIAF-531
1	961.3 cR from top of Chr1 link	2479	WIAF   WIAF-75
30	962.8 cR from top of Chr1 link	2637	WIAF   WIAF-297
1	969.0 cR from top of Chr1 link	3114	WIAF   WIAF-1015
1	980.4 cR from top of Chr1 link	2976	WIAF   WIAF-876
1	980.4 cR from top of Chr1 link	2977	WIAF   WIAF-877
1	996.9 cR from top of Chr1 link	2897	WIAF   WIAF-795
35	998.5 cR from top of Chr1 link	2541	WIAF   WIAF-155
1		4221	MARSHFIELD   MID-13
1		4222	MARSHFIELD   MID-14
1		3996	SHGC/AFFYMETRIX   SNP-SHGC-10870
1		4004	SHGC/AFFYMETRIX   SNP-SHGC-12999
40	1	4155	SHGC/AFFYMETRIX   SNP-SHGC-14385
1		4082	SHGC/AFFYMETRIX   SNP-SHGC-16847
1		4098	SHGC/AFFYMETRIX   SNP-SHGC-18912
1		4037	SHGC/AFFYMETRIX   SNP-SHGC-8109
1		4041	SHGC/AFFYMETRIX   SNP-SHGC-8491
45	1	4043	SHGC/AFFYMETRIX   SNP-SHGC-8995
1		4049	SHGC/AFFYMETRIX   SNP-SHGC-9374
1		3117	WIAF   WIAF-1018
1		3203	WIAF   WIAF-1546
1		3204	WIAF   WIAF-1547
50	1	3222	WIAF   WIAF-1610

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
1		3315	WIAF   WIAF-1768
1		3432	WIAF   WIAF-1887
1		3515	WIAF   WIAF-1970
1		3578	WIAF   WIAF-2033
5	1	1519	WIAF   WIAF-3458
	1	3887	WIAF   WIAF-3948
	1	3914	WIAF   WIAF-3998
	1	3915	WIAF   WIAF-4000
	1	2955	WIAF   WIAF-854
10	1	2969	WIAF   WIAF-869
	2 0.00 cR from top of Chr2 link a	2010	WIAF   WIAF-1492
	2 6 cM	4326	UWGC   145
15	2 6.00 cR from top of Chr2 link a	706	WIAF   WIAF-1363
	2 6.00 cR from top of Chr2 link a	1446	WIAF   WIAF-3376
	2 9.40 cR from top of Chr2 link a	2676	WIAF   WIAF-358
	2 12.10 cR from top of Chr2 link	3383	WIAF   WIAF-1836
	2 12.10 cR from top of Chr2 link	3384	WIAF   WIAF-1837
	2 24.50 cR from top of Chr2 link	1276	WIAF   WIAF-2163
20	2 32.90 cR from top of Chr2 link	1334	WIAF   WIAF-2224
	2 36.60 cR from top of Chr2 link	1201	WIAF   WIAF-2088
	2 40.20 cR from top of Chr2 link	1203	WIAF   WIAF-2090
	2 41.5 cR from top of Chr2 link a	2517	WIAF   WIAF-125
	2 44.40 cR from top of Chr2 link	698	WIAF   WIAF-1268
25	2 44.6 cR from top of Chr2 link a	2750	WIAF   WIAF-469
	2 46.00 cR from top of Chr2 link	1228	WIAF   WIAF-2115
	2 46.1 cR from top of Chr2 link a	3385	WIAF   WIAF-1839
	2 47.90 cR from top of Chr2 link	3236	WIAF   WIAF-1645
	2 47.90 cR from top of Chr2 link	3237	WIAF   WIAF-1646
30	2 50.30 cR from top of Chr2 link	1420	WIAF   WIAF-3348
	2 50.70 cR from top of Chr2 link	1129	WIAF   WIAF-1573
	2 51.10 cR from top of Chr2 link	2925	WIAF   WIAF-824
	2 51.40 cR from top of Chr2 link	3223	WIAF   WIAF-1612
	2 51.40 cR from top of Chr2 link	1311	WIAF   WIAF-2200
35	2 54.7 cR from top of Chr2 link a	3033	WIAF   WIAF-933
	2 55.00 cR from top of Chr2 link	1975	WIAF   WIAF-3914
	2 64.90 cR from top of Chr2 link	3345	WIAF   WIAF-1798
	2 64.90 cR from top of Chr2 link	1529	WIAF   WIAF-3468
	2 66.80 cR from top of Chr2 link	2014	WIAF   WIAF-1508
40	2 69.00 cR from top of Chr2 link	1177	WIAF   WIAF-1705
	2 70.30 cR from top of Chr2 link	1920	WIAF   WIAF-3859
	2 70.30 cR from top of Chr2 link	1922	WIAF   WIAF-3861
	2 70.60 cR from top of Chr2 link	2023	WIAF   WIAF-1562
	2 71.70 cR from top of Chr2 link	1347	WIAF   WIAF-3250
45	2 76.60 cR from top of Chr2 link	1104	WIAF   WIAF-1528
	2 79.70 cR from top of Chr2 link	1257	WIAF   WIAF-2144
	2 82.20 cR from top of Chr2 link	1694	WIAF   WIAF-3633
	2 84.8 cR from top of Chr2 link a	2850	WIAF   WIAF-714
	2 87.10 cR from top of Chr2 link	1599	WIAF   WIAF-3538
50	2 89.70 cR from top of Chr2 link	1280	WIAF   WIAF-2167

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	2 89.70 cR from top of Chr2 link	1594	WIAF   WIAF-3533
	2 90.10 cR from top of Chr2 link	692	WIAF   WIAF-1226
	2 91.60 cR from top of Chr2 link	1412	WIAF   WIAF-3333
	2 92.2 cR from top of Chr2 link	3103	WIAF   WIAF-1004
5	2 92.20 cR from top of Chr2 link	1423	WIAF   WIAF-3351
	2 93.80 cR from top of Chr2 link	1243	WIAF   WIAF-2130
	2 96.00 cR from top of Chr2 link	1162	WIAF   WIAF-1665
	2 106.10 cR from top of Chr2 lin	3324	WIAF   WIAF-1777
	2 106.10 cR from top of Chr2 lin	1955	WIAF   WIAF-3894
10	2 110.00 cR from top of Chr2 lin	1684	WIAF   WIAF-3623
	2 112.40 cR from top of Chr2 lin	1611	WIAF   WIAF-3550
	2 112.40 cR from top of Chr2 lin	1613	WIAF   WIAF-3552
	2 115.30 cR from top of Chr2 lin	1286	WIAF   WIAF-2173
	2 115.30 cR from top of Chr2 lin	1287	WIAF   WIAF-2174
15	2 117.60 cR from top of Chr2 lin	3509	WIAF   WIAF-1964
	2 117.60 cR from top of Chr2 lin	3510	WIAF   WIAF-1965
	2 118.60 cR from top of Chr2 lin	1327	WIAF   WIAF-2217
	2 118.80 cR from top of Chr2 lin	3458	WIAF   WIAF-1913
	2 118.80 cR from top of Chr2 lin	3459	WIAF   WIAF-1914
20	2 118.80 cR from top of Chr2 lin	3460	WIAF   WIAF-1915
	2 119.20 cR from top of Chr2 lin	2017	WIAF   WIAF-1518
	2 119.30 cR from top of Chr2 lin	1653	WIAF   WIAF-3592
	2 122.40 cR from top of Chr2 lin	702	WIAF   WIAF-1311
	2 123.10 cR from top of Chr2 lin	1503	WIAF   WIAF-3442
25	2 123.10 cR from top of Chr2 lin	1504	WIAF   WIAF-3443
	2 123.4 cR from top of Chr2 link	2638	WIAF   WIAF-304
	2 124.50 cR from top of Chr2 lin	3014	WIAF   WIAF-914
	2 134.30 cR from top of Chr2 lin	1091	WIAF   WIAF-1467
	2 134.30 cR from top of Chr2 lin	1915	WIAF   WIAF-3854
30	2 135.80 cR from top of Chr2 lin	1724	WIAF   WIAF-3663
	2 149.50 cR from top of Chr2 lin	1617	WIAF   WIAF-3556
	2 152.6 cR from top of Chr2 link	2284	WIAF   WIAF-757
	2 158.40 cR from top of Chr2 lin	3208	WIAF   WIAF-1559
	2 158.40 cR from top of Chr2 lin	3209	WIAF   WIAF-1560
35	2 159.40 cR from top of Chr2 lin	1824	WIAF   WIAF-3763
	2 162.90 cR from top of Chr2 lin	1699	WIAF   WIAF-3638
	2 164.60 cR from top of Chr2 lin	1947	WIAF   WIAF-3886
	2 166.4 cR from top of Chr2 link	3054	WIAF   WIAF-955
	2 166.50 cR from top of Chr2 lin	3173	WIAF   WIAF-1487
40	2 169.10 cR from top of Chr2 lin	1455	WIAF   WIAF-3388
	2 180.30 cR from top of Chr2 lin	1368	WIAF   WIAF-3271
	2 188.20 cR from top of Chr2 lin	1728	WIAF   WIAF-3667
	2 188.40 cR from top of Chr2 lin	3431	WIAF   WIAF-1886
	2 188.60 cR from top of Chr2 lin	1206	WIAF   WIAF-2093
45	2 188.70 cR from top of Chr2 lin	1356	WIAF   WIAF-3259
	2 190.80 cR from top of Chr2 lin	1677	WIAF   WIAF-3616
	2 191.20 cR from top of Chr2 lin	2025	WIAF   WIAF-1570
	2 191.20 cR from top of Chr2 lin	1164	WIAF   WIAF-1675
	2 191.40 cR from top of Chr2 lin	1509	WIAF   WIAF-3448
50	2 191.50 cR from top of Chr2 lin	2636	WIAF   WIAF-296

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	2 192.9 cR from top of Chr2 link	2454	WIAF   WIAF-45
	2 192.9 cR from top of Chr2 link	2455	WIAF   WIAF-46
	2 195.10 cR from top of Chr2 lin	1193	WIAF   WIAF-2080
	2 200.30 cR from top of Chr2 lin	1248	WIAF   WIAF-2135
5	2 200.40 cR from top of Chr2 lin	1619	WIAF   WIAF-3558
	2 201.5 cR from top of Chr2 link	2968	WIAF   WIAF-868
	2 202.7 cR from top of Chr2 link	2503	WIAF   WIAF-107
	2 208.30 cR from top of Chr2 lin	1676	WIAF   WIAF-3615
	2 208.30 cR from top of Chr2 lin	1678	WIAF   WIAF-3617
10	2 213.00 cR from top of Chr2 lin	3813	WIAF   WIAF-2565
	2 214.50 cR from top of Chr2 lin	3487	WIAF   WIAF-1942
	2 219.30 cR from top of Chr2 lin	1288	WIAF   WIAF-2175
	2 219.30 cR from top of Chr2 lin	1289	WIAF   WIAF-2176
	2 220.10 cR from top of Chr2 lin	1736	WIAF   WIAF-3675
15	2 221.1 cR from top of Chr2 link	909	WIAF   WIAF-1184
	2 221.1 cR from top of Chr2 link	1046	WIAF   WIAF-4141
	2 221.50 cR from top of Chr2 lin	3310	WIAF   WIAF-1763
	2 222.6 cR from top of Chr2 link	3321	WIAF   WIAF-1774
	2 223.40 cR from top of Chr2 lin	3512	WIAF   WIAF-1967
20	2 229.80 cR from top of Chr2 lin	1510	WIAF   WIAF-3449
	2 229.80 cR from top of Chr2 lin	1511	WIAF   WIAF-3450
	2 234.50 cR from top of Chr2 lin	1523	WIAF   WIAF-3462
	2 236.10 cR from top of Chr2 lin	2020	WIAF   WIAF-1526
	2 236.10 cR from top of Chr2 lin	1844	WIAF   WIAF-3783
25	2 236.10 cR from top of Chr2 lin	1846	WIAF   WIAF-3785
	2 240.20 cR from top of Chr2 lin	1384	WIAF   WIAF-3289
	2 242.40 cR from top of Chr2 lin	1663	WIAF   WIAF-3602
	2 246.10 cR from top of Chr2 lin	1303	WIAF   WIAF-2192
	2 247.10 cR from top of Chr2 lin	713	WIAF   WIAF-1451
30	2 247.20 cR from top of Chr2 lin	1502	WIAF   WIAF-3441
	2 253.00 cR from top of Chr2 lin	1309	WIAF   WIAF-2198
	2 269.50 cR from top of Chr2 lin	1750	WIAF   WIAF-3689
	2 272.50 cR from top of Chr2 lin	1534	WIAF   WIAF-3473
	2 272.50 cR from top of Chr2 lin	1702	WIAF   WIAF-3641
35	2 272.60 cR from top of Chr2 lin	2875	WIAF   WIAF-773
	2 278.8 cR from top of Chr2 link	3825	WIAF   WIAF-2590
	2 285.6 cR from top of Chr2 link	3539	WIAF   WIAF-1994
	2 285.7 cR from top of Chr2 link	3849	WIAF   WIAF-2635
	2 287.2 cR from top of Chr2 link	3587	WIAF   WIAF-2052
40	2 287.2 cR from top of Chr2 link	1071	WIAF   WIAF-4203
	2 290.4 cR from top of Chr2 link	2697	WIAF   WIAF-383
	2 293.7 cR from top of Chr2 link	2154	WIAF   WIAF-486
	2 293.7 cR from top of Chr2 link	2155	WIAF   WIAF-487
	2 300.1 cR from top of Chr2 link	2923	WIAF   WIAF-822
45	2 318.2 cR from top of Chr2 link	966	WIAF   WIAF-1371
	2 325.6 cR from top of Chr2 link	4081	SHGC/AFFYMETRIX   SNP-SHGC-16802
	2 341.6 cR from top of Chr2 link	2281	WIAF   WIAF-751
	2 375.3 cR from top of Chr2 link	2863	WIAF   WIAF-760
	2 742.6 cR from top of Chr2 link	2213	WIAF   WIAF-635
50	2 750.0 cR from top of Chr2 link	2639	WIAF   WIAF-305

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
2	750.1 cR from top of Chr2 link	2954	WIAF   WIAF-853
2	758.7 cR from top of Chr2 link	893	WIAF   WIAF-1140
2	758.7 cR from top of Chr2 link	1059	WIAF   WIAF-4175
2	780.9 cR from top of Chr2 link	2253	WIAF   WIAF-701
5	2 783.9 cR from top of Chr2 link	2673	WIAF   WIAF-353
	2 796.0 cR from top of Chr2 link	2493	WIAF   WIAF-91
	2 824.7 cR from top of Chr2 link	2472	WIAF   WIAF-66
	2 838.5 cR from top of Chr2 link	2194	WIAF   WIAF-594
	2 854.9 cR from top of Chr2 link	2209	WIAF   WIAF-629
10	2 881.0 cR from top of Chr2 link	2878	WIAF   WIAF-776
	2 900.2 cR from top of Chr2 link	3858	WIAF   WIAF-2647
	2 902.1 cR from top of Chr2 link	2661	WIAF   WIAF-337
	2 910.6 cR from top of Chr2 link	2961	WIAF   WIAF-860
	2 910.6 cR from top of Chr2 link	2962	WIAF   WIAF-861
15	2 910.6 cR from top of Chr2 link	2963	WIAF   WIAF-862
	2 915.7 cR from top of Chr2 link	2500	WIAF   WIAF-99
	2 920.4 cR from top of Chr2 link	726	WIAF   WIAF-1066
	2 931.1 cR from top of Chr2 link	2104	WIAF   WIAF-177
	2 952.3 cR from top of Chr2 link	2516	WIAF   WIAF-124
20	2 956.7 cR from top of Chr2 link	2629	WIAF   WIAF-289
	2 961.8 cR from top of Chr2 link	3109	WIAF   WIAF-1010
	2 981.1 cR from top of Chr2 link	2470	WIAF   WIAF-64
	2 986.9 cR from top of Chr2 link	2125	WIAF   WIAF-300
	2 986.9 cR from top of Chr2 link	2126	WIAF   WIAF-301
25	2 1009.4 cR from top of Chr2 lin	2978	WIAF   WIAF-878
	2 1026.1 cR from top of Chr2 lin	3871	WIAF   WIAF-2670
	2 1026.1 cR from top of Chr2 lin	3872	WIAF   WIAF-2671
	2 1074.0 cR from top of Chr2 lin	2738	WIAF   WIAF-450
	2 1089.0 cR from top of Chr2 lin	2052	WIAF   WIAF-1700
30	2 1092.0 cR from top of Chr2 lin	3474	WIAF   WIAF-1929
	2 1092.0 cR from top of Chr2 lin	3475	WIAF   WIAF-1930
	2 1104.9 cR from top of Chr2 lin	3309	WIAF   WIAF-1762
		4223	MARSHFIELD   MID-15
		4224	MARSHFIELD   MID-16
35	2	3962	SHGC/AFFYMETRIX   SNP-SHGC-11130
	2	4069	SHGC/AFFYMETRIX   SNP-SHGC-13615
	2	3967	SHGC/AFFYMETRIX   SNP-SHGC-13867
	2	3968	CHGC/AFFYMETRIX   SNP-SHGC-13934
	2	4164	SHGC/AFFYMETRIX   SNP-SHGC-15247
40	2	4074	SHGC/AFFYMETRIX   SNP-SHGC-15661
	2	4087	SHGC/AFFYMETRIX   SNP-SHGC-17089
	2	4016	SHGC/AFFYMETRIX   SNP-SHGC-3987
	2	4040	SHGC/AFFYMETRIX   SNP-SHGC-8478
	2	4044	SHGC/AFFYMETRIX   SNP-SHGC-9017
45	2	4048	SHGC/AFFYMETRIX   SNP-SHGC-9366
	2	3122	WIAF   WIAF-1023
	2	3130	WIAF   WIAF-1031
	2	3159	WIAF   WIAF-1458
	2	1218	WIAF   WIAF-2105
50	2	1231	WIAF   WIAF-2118

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	2	1253	WIAF   WIAF-2140
	2	1254	WIAF   WIAF-2141
	2	3672	WIAF   WIAF-2400
	2	3683	WIAF   WIAF-2411
5	2	3705	WIAF   WIAF-2433
	2	3781	WIAF   WIAF-2509
	2	3782	WIAF   WIAF-2510
	2	2447	WIAF   WIAF-35
	2	2448	WIAF   WIAF-36
10	2	2449	WIAF   WIAF-37
	2	2480	WIAF   WIAF-76
	2	3080	WIAF   WIAF-981
	2	3097	WIAF   WIAF-998
	2	3098	WIAF   WIAF-999
15			
	3 12.90 cR from top of Chr3 link	1522	WIAF   WIAF-3461
	3 12.90 cR from top of Chr3 link	1524	WIAF   WIAF-3463
	3 14.5 cR from top of Chr3 linka	2098	WIAF   WIAF-144
	3 18.4 cR from top of Chr3 linka	3339	WIAF   WIAF-1792
20	3 18.4 cR from top of Chr3 linka	3340	WIAF   WIAF-1793
	3 19.3 cR from top of Chr3 linka	2244	WIAF   WIAF-685
	3 33.50 cR from top of Chr3 link	3811	WIAF   WIAF-2563
	3 33.50 cR from top of Chr3 link	1926	WIAF   WIAF-3865
	3 36.50 cR from top of Chr3 link	2886	WIAF   WIAF-784
25	3 36.90 cR from top of Chr3 link	1893	WIAF   WIAF-3832
	3 37.90 cR from top of Chr3 link	1142	WIAF   WIAF-1605
	3 43.20 cR from top of Chr3 link	1494	WIAF   WIAF-3433
	3 44.1 cR from top of Chr3 linka	2939	WIAF   WIAF-838
	3 45.30 cR from top of Chr3 link	3491	WIAF   WIAF-1946
30	3 46.90 cR from top of Chr3 link	3312	WIAF   WIAF-1765
	3 49.00 cR from top of Chr3 link	1449	WIAF   WIAF-3380
	3 49.00 cR from top of Chr3 link	1450	WIAF   WIAF-3382
	3 51.7 cR from top of Chr3 linka	2191	WIAF   WIAF-587
	3 54.9 cR from top of Chr3 linka	2456	WIAF   WIAF-47
35	3 55.0 cR from top of Chr3 linka	3863	WIAF   WIAF-2656
	3 55.40 cR from top of Chr3 link	3471	WIAF   WIAF-1926
	3 55.60 cR from top of Chr3 link	3336	WIAF   WIAF-1789
	3 56.8 cR from top of Chr3 linka	2508	WIAF   WIAF-114
	3 56.8 cR from top of Chr3 linka	2509	WIAF   WIAF-115
40	3 57.80 cR from top of Chr3 link	2037	WIAF   WIAF-1617
	3 57.80 cR from top of Chr3 link	1825	WIAF   WIAF-3764
	3 57.80 cR from top of Chr3 link	2707	WIAF   WIAF-398
	3 58.00 cR from top of Chr3 link	2984	WIAF   WIAF-884
	3 66.40 cR from top of Chr3 link	1308	WIAF   WIAF-2197
45	3 66.80 cR from top of Chr3 link	3225	WIAF   WIAF-1624
	3 66.80 cR from top of Chr3 link	3483	WIAF   WIAF-1938
	3 67.20 cR from top of Chr3 link	683	WIAF   WIAF-1074
	3 67.50 cR from top of Chr3 link	3245	WIAF   WIAF-1655
	3 67.50 cR from top of Chr3 link	1602	WIAF   WIAF-3541
50	3 72.1 cR from top of Chr3 linka	3308	WIAF   WIAF-1761

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
3	72.30 cR from top of Chr3 link	3193	WIAF   WIAF-1522
3	72.30 cR from top of Chr3 link	3194	WIAF   WIAF-1523
3	72.40 cR from top of Chr3 link	826	WIAF   WIAF-1489
3	72.60 cR from top of Chr3 link	3410	WIAF   WIAF-1865
5	72.8 cR from top of Chr3 linka	2622	WIAF   WIAF-281
3	73.3 cR from top of Chr3 linka	3868	WIAF   WIAF-2663
3	74.00 cR from top of Chr3 link	1595	WIAF   WIAF-3534
3	77.40 cR from top of Chr3 link	1690	WIAF   WIAF-3629
	80 cM	4314	UWGC   133
10	80.80 cR from top of Chr3 link	3378	WIAF   WIAF-1831
3	92.80 cR from top of Chr3 link	1452	WIAF   WIAF-3385
3	96.60 cR from top of Chr3 link	1770	WIAF   WIAF-3709
3	111.00 cR from top of Chr3 lin	3341	WIAF   WIAF-1794
3	111.10 cR from top of Chr3 lin	3189	WIAF   WIAF-1512
15	111.10 cR from top of Chr3 lin	3190	WIAF   WIAF-1513
3	111.40 cR from top of Chr3 lin	1956	WIAF   WIAF-3895
3	122.30 cR from top of Chr3 lin	1549	WIAF   WIAF-3488
3	124.00 cR from top of Chr3 lin	1182	WIAF   WIAF-1714
3	126.3 cR from top of Chr3 link	2854	WIAF   WIAF-741
20	126.8 cR from top of Chr3 link	3123	WIAF   WIAF-1024
3	126.90 cR from top of Chr3 lin	1454	WIAF   WIAF-3387
3	129.30 cR from top of Chr3 lin	1395	WIAF   WIAF-3300
3	131.30 cR from top of Chr3 lin	1923	WIAF   WIAF-3862
3	134.60 cR from top of Chr3 lin	1259	WIAF   WIAF-2146
25	134.9 cR from top of Chr3 link	917	WIAF   WIAF-1207
3	134.9 cR from top of Chr3 link	918	WIAF   WIAF-1208
3	134.9 cR from top of Chr3 link	919	WIAF   WIAF-1209
3	136.00 cR from top of Chr3 lin	1931	WIAF   WIAF-3870
3	138.00 cR from top of Chr3 lin	3228	WIAF   WIAF-1629
30	138.30 cR from top of Chr3 lin	1963	WIAF   WIAF-3902
3	138.40 cR from top of Chr3 lin	1725	WIAF   WIAF-3664
3	140.70 cR from top of Chr3 lin	1092	WIAF   WIAF-1473
3	141.0 cR from top of Chr3 link	3147	WIAF   WIAF-1048
3	141.20 cR from top of Chr3 lin	1970	WIAF   WIAF-3909
35	142.20 cR from top of Chr3 lin	1229	WIAF   WIAF-2116
3	142.40 cR from top of Chr3 lin	1187	WIAF   WIAF-2074
3	143.80 cR from top of Chr3 lin	3263	WIAF   WIAF-1702
3	143.80 cR from top of Chr3 lin	3264	WIAF   WIAF-1703
3	143.90 cR from top of Chr3 lin	1195	WIAF   WIAF-2082
40	144.20 cR from top of Chr3 lin	1158	WIAF   WIAF-1656
3	144.70 cR from top of Chr3 lin	1722	WIAF   WIAF-3661
3	147.8 cR from top of Chr3 link	2572	WIAF   WIAF-202
3	151.20 cR from top of Chr3 lin	1152	WIAF   WIAF-1636
3	151.20 cR from top of Chr3 lin	1153	WIAF   WIAF-1637
45	153.80 cR from top of Chr3 lin	1890	WIAF   WIAF-3829
3	156.30 cR from top of Chr3 lin	1716	WIAF   WIAF-3655
3	156.60 cR from top of Chr3 lin	1734	WIAF   WIAF-3673
3	164.20 cR from top of Chr3 lin	3296	WIAF   WIAF-1749
3	164.20 cR from top of Chr3 lin	1629	WIAF   WIAF-3568
50	166.0 cR from top of Chr3 link	2898	WIAF   WIAF-796

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
3	170.20 cR from top of Chr3 lin	1852	WIAF   WIAF-3791
3	171.7 cR from top of Chr3 link	2917	WIAF   WIAF-816
3	173.30 cR from top of Chr3 lin	1351	WIAF   WIAF-3254
3	173.50 cR from top of Chr3 lin	1407	WIAF   WIAF-3327
5	3 186.60 cR from top of Chr3 lin	1819	WIAF   WIAF-3758
	3 186.7 cR from top of Chr3 link	2571	WIAF   WIAF-201
	3 187.60 cR from top of Chr3 lin	1305	WIAF   WIAF-2194
	3 187.9 cR from top of Chr3 link	2114	WIAF   WIAF-236
	3 189.80 cR from top of Chr3 lin	1463	WIAF   WIAF-3398
10	3 195.7 cR from top of Chr3 link	3847	WIAF   WIAF-2626
	3 228.30 cR from top of Chr3 lin	1887	WIAF   WIAF-3826
	3 228.30 cR from top of Chr3 lin	1888	WIAF   WIAF-3827
	3 228.4 cR from top of Chr3 link	3472	WIAF   WIAF-1927
15	3 228.4 cR from top of Chr3 link	3473	WIAF   WIAF-1928
	3 228.60 cR from top of Chr3 lin	1161	WIAF   WIAF-1664
	3 233.00 cR from top of Chr3 lin	1383	WIAF   WIAF-3288
	3 233.00 cR from top of Chr3 lin	1470	WIAF   WIAF-3405
	3 233.00 cR from top of Chr3 lin	1471	WIAF   WIAF-3406
	3 233.00 cR from top of Chr3 lin	1587	WIAF   WIAF-3526
20	3 233.00 cR from top of Chr3 lin	1627	WIAF   WIAF-3566
	3 233.8 cR from top of Chr3 link	3446	WIAF   WIAF-1901
	3 239.5 cR from top of Chr3 link	1032	WIAF   WIAF-4092
	3 240.4 cR from top of Chr3 link	2234	WIAF   WIAF-669
	3 240.4 cR from top of Chr3 link	2235	WIAF   WIAF-670
25	3 269.6 cR from top of Chr3 link	868	WIAF   WIAF-1081
	3 269.6 cR from top of Chr3 link	1003	WIAF   WIAF-4026
	3 463.3 cR from top of Chr3 link	2342	WIAF   WIAF-2572
	3 477.2 cR from top of Chr3 link	4560	HU-CHINA   1-1176-2
	3 477.2 cR from top of Chr3 link	4587	HU-CHINA   3-1176
30	3 477.2 cR from top of Chr3 link	741	WIAF   WIAF-1176
	3 533.1 cR from top of Chr3 link	3839	WIAF   WIAF-2612
	3 534.4 cR from top of Chr3 link	2855	WIAF   WIAF-745
	3 546.0 cR from top of Chr3 link	3085	WIAF   WIAF-986
	3 552.8 cR from top of Chr3 link	1753	WIAF   WIAF-3692
35	3 569.6 cR from top of Chr3 link	3354	WIAF   WIAF-1807
	3 569.6 cR from top of Chr3 link	2440	WIAF   WIAF-25
	3 604.7 cR from top of Chr3 link	2358	WIAF   WIAF-2606
	3 611.1 cR from top of Chr3 link	2905	WIAF   WIAF-803
	3 616.0 cR from top of Chr3 link	788	WIAF   WIAF-2056
40	3 640.6 cR from top of Chr3 link	2185	WIAF   WIAF-568
	3 640.6 cR from top of Chr3 link	2186	WIAF   WIAF-569
	3 672.1 cR from top of Chr3 link	2830	WIAF   WIAF-650
	3 672.1 cR from top of Chr3 link	2831	WIAF   WIAF-651
	3 680.5 cR from top of Chr3 link	2379	WIAF   WIAF-2664
45	3 680.5 cR from top of Chr3 link	2380	WIAF   WIAF-2665
	3 690.2 cR from top of Chr3 link	2656	WIAF   WIAF-330
	3 718.0 cR from top of Chr3 link	2207	WIAF   WIAF-625
	3 718.0 cR from top of Chr3 link	2208	WIAF   WIAF-626
	3 718.5 cR from top of Chr3 link	2217	WIAF   WIAF-639
50	3 775.9 cR from top of Chr3 link	2461	WIAF   WIAF-52

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
3	791.4 cR from top of Chr3 link	2574	WIAF   WIAF-204
3	792.2 cR from top of Chr3 link	1256	WIAF   WIAF-2143
3	793.4 cR from top of Chr3 link	2948	WIAF   WIAF-847
3	793.7 cR from top of Chr3 link	2779	WIAF   WIAF-523
5	796.7 cR from top of Chr3 link	2788	WIAF   WIAF-542
3	802.4 cR from top of Chr3 link	2173	WIAF   WIAF-543
3	808.9 cR from top of Chr3 link	2246	WIAF   WIAF-690
3	838.9 cR from top of Chr3 link	2604	WIAF   WIAF-249
3	838.9 cR from top of Chr3 link	2605	WIAF   WIAF-250
10	842.9 cR from top of Chr3 link	2703	WIAF   WIAF-392
3	848.1 cR from top of Chr3 link	2630	WIAF   WIAF-290
3	848.1 cR from top of Chr3 link	2631	WIAF   WIAF-291
3	848.1 cR from top of Chr3 link	2632	WIAF   WIAF-292
3	868.2 cR from top of Chr3 link	3814	WIAF   WIAF-2568
15	868.6 cR from top of Chr3 link	3366	WIAF   WIAF-1819
3	879.8 cR from top of Chr3 link	224	KWOK   D3S2344-1
3	879.8 cR from top of Chr3 link	225	KWOK   D3S2344-2
3	879.8 cR from top of Chr3 link	766	WIAF   WIAF-1365
3	896.5 cR from top of Chr3 link	3333	WIAF   WIAF-1786
20	897.8 cR from top of Chr3 link	3451	WIAF   WIAF-1906
3	903.2 cR from top of Chr3 link	3360	WIAF   WIAF-1813
3	907.0 cR from top of Chr3 link	2513	WIAF   WIAF-119
3	907.0 cR from top of Chr3 link	2514	WIAF   WIAF-120
3	917.9 cR from top of Chr3 link	3078	WIAF   WIAF-979
25	918.0 cR from top of Chr3 link	2543	WIAF   WIAF-162
3	921.8 cR from top of Chr3 link	3106	WIAF   WIAF-1007
		4225	MARSHFIELD   MID-17
		3998	SHGC/AFFYMETRIX   SNP-SHGC-11665
		3999	SHGC/AFFYMETRIX   SNP-SHGC-1204
30	3	4138	SHGC/AFFYMETRIX   SNP-SHGC-13087
		4067	SHGC/AFFYMETRIX   SNP-SHGC-13482
		4147	SHGC/AFFYMETRIX   SNP-SHGC-14087
		4151	SHGC/AFFYMETRIX   SNP-SHGC-14182
		4156	SHGC/AFFYMETRIX   SNP-SHGC-14457
35	3	4162	SHGC/AFFYMETRIX   SNP-SHGC-14769
		3970	SHGC/AFFYMETRIX   SNP-SHGC-16777
		4089	SHGC/AFFYMETRIX   SNP-SHGC-17103
		4097	SHGC/AFFYMETRIX   SNP-SHGC-18889
40	3	4106	SHGC/AFFYMETRIX   SNP-SHGC-32258
		4012	SHGC/AFFYMETRIX   SNP-SHGC-3249
		3974	SHGC/AFFYMETRIX   SNP-SHGC-33980
		4107	SHGC/AFFYMETRIX   SNP-SHGC-35481
		4035	SHGC/AFFYMETRIX   SNP-SHGC-7204
		3144	WIAF   WIAF-1045
45	3	3146	WIAF   WIAF-1047
		3530	WIAF   WIAF-1985
		3740	WIAF   WIAF-2468
		2061	WIAF   WIAF-2547
		1500	WIAF   WIAF-3439
50	3	1673	WIAF   WIAF-3612

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
3		3925	WIAF   WIAF-4013
3		2996	WIAF   WIAF-896
3		3006	WIAF   WIAF-906
3		3017	WIAF   WIAF-917
5	3	3048	WIAF   WIAF-949
10	4 3.70 cR from top of Chr4 linka	1204	WIAF   WIAF-2091
	4 3.70 cR from top of Chr4 linka	1919	WIAF   WIAF-3858
	4 4.4 cR from top of Chr4 linkag	3866	WIAF   WIAF-2660
10	4 4.70 cR from top of Chr4 linka	3215	WIAF   WIAF-1591
	4 4.70 cR from top of Chr4 linka	3216	WIAF   WIAF-1592
	4 4.70 cR from top of Chr4 linka	3217	WIAF   WIAF-1593
	4 4.70 cR from top of Chr4 linka	1210	WIAF   WIAF-2097
	4 5.30 cR from top of Chr4 linka	1120	WIAF   WIAF-1555
15	4 8.60 cR from top of Chr4 linka	3233	WIAF   WIAF-1639
	4 15.00 cR from top of Chr4 link	1332	WIAF   WIAF-2222
	4 18.1 cR from top of Chr4 linka	3809	WIAF   WIAF-2561
	4 18.70 cR from top of Chr4 link	1307	WIAF   WIAF-2196
	4 19.8 cR from top of Chr4 linka	3503	WIAF   WIAF-1958
20	4 22.00 cR from top of Chr4 link	3250	WIAF   WIAF-1668
	4 26.80 cR from top of Chr4 link	1811	WIAF   WIAF-3750
	4 26.80 cR from top of Chr4 link	1814	WIAF   WIAF-3753
	4 27.7 cR from top of Chr4 linka	2070	WIAF   WIAF-2557
	4 28.20 cR from top of Chr4 link	3161	WIAF   WIAF-1464
25	4 28.90 cR from top of Chr4 link	3555	WIAF   WIAF-2010
	4 29.80 cR from top of Chr4 link	3507	WIAF   WIAF-1962
	4 29.80 cR from top of Chr4 link	3508	WIAF   WIAF-1963
	4 35.40 cR from top of Chr4 link	3163	WIAF   WIAF-1466
	4 36.90 cR from top of Chr4 link	1520	WIAF   WIAF-3459
30	4 39.3 cR from top of Chr4 linka	2076	WIAF   WIAF-14
	4 43.50 cR from top of Chr4 link	707	WIAF   WIAF-1395
	4 45.9 cR from top of Chr4 linka	3003	WIAF   WIAF-903
	4 51.00 cR from top of Chr4 link	3470	WIAF   WIAF-1925
	4 51.90 cR from top of Chr4 link	2021	WIAF   WIAF-1527
35	4 55.2 cR from top of Chr4 linka	231	KWOK   D4S2341
	4 55.2 cR from top of Chr4 linka	2092	WIAF   WIAF-109
	4 55.2 cR from top of Chr4 linka	780	WIAF   WIAF-1433
	4 55.2 cR from top of Chr4 linka	2340	WIAF   WIAF-2566
	4 55.2 cR from top of Chr4 linka	806	WIAF   WIAF-4142
40	4 58.40 cR from top of Chr4 link	1475	WIAF   WIAF-3412
	4 61.8 cR from top of Chr4 linka	2210	WIAF   WIAF-630
	4 65.2 cR from top of Chr4 linka	2129	WIAF   WIAF-316
	4 74.30 cR from top of Chr4 link	1461	WIAF   WIAF-3395
	4 78.50 cR from top of Chr4 link	3131	WIAF   WIAF-1032
45	4 80.70 cR from top of Chr4 link	672	WIAF   WIAF-1370
	4 91.50 cR from top of Chr4 link	1145	WIAF   WIAF-1611
	4 91.70 cR from top of Chr4 link	1433	WIAF   WIAF-3361
	4 96.60 cR from top of Chr4 link	1097	WIAF   WIAF-1485
	4 98.5 cR from top of Chr4 linka	3520	WIAF   WIAF-1975
50	4 98.5 cR from top of Chr4 linka	3521	WIAF   WIAF-1976

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
4	98.80 cR from top of Chr4 link	1226	WIAF   WIAF-2113
4	98.80 cR from top of Chr4 link	1227	WIAF   WIAF-2114
4	106.40 cR from top of Chr4 lin	1826	WIAF   WIAF-3765
4	108.70 cR from top of Chr4 lin	703	WIAF   WIAF-1333
5	112.10 cR from top of Chr4 lin	1252	WIAF   WIAF-2139
4	120.20 cR from top of Chr4 lin	1304	WIAF   WIAF-2193
4	121.00 cR from top of Chr4 lin	2019	WIAF   WIAF-1524
4	121.40 cR from top of Chr4 lin	3165	WIAF   WIAF-1469
4	121.50 cR from top of Chr4 lin	1348	WIAF   WIAF-3251
10	121.50 cR from top of Chr4 lin	1871	WIAF   WIAF-3810
4	121.50 cR from top of Chr4 lin	1872	WIAF   WIAF-3811
4	122.60 cR from top of Chr4 lin	1575	WIAF   WIAF-3514
4	122.60 cR from top of Chr4 lin	1576	WIAF   WIAF-3515
4	124.40 cR from top of Chr4 lin	1354	WIAF   WIAF-3257
15	125.80 cR from top of Chr4 lin	1879	WIAF   WIAF-3818
4	125.80 cR from top of Chr4 lin	1882	WIAF   WIAF-3821
4	126.10 cR from top of Chr4 lin	1953	WIAF   WIAF-3892
4	128.3 cR from top of Chr4 link	2467	WIAF   WIAF-60
4	139.80 cR from top of Chr4 lin	1906	WIAF   WIAF-3845
20	140.70 cR from top of Chr4 lin	1966	WIAF   WIAF-3905
4	141.70 cR from top of Chr4 lin	1466	WIAF   WIAF-3401
4	144.1 cR from top of Chr4 link	3129	WIAF   WIAF-1030
4	145.50 cR from top of Chr4 lin	1093	WIAF   WIAF-1475
4	146.00 cR from top of Chr4 lin	2902	WIAF   WIAF-800
25	148.40 cR from top of Chr4 lin	1135	WIAF   WIAF-1589
4	148.60 cR from top of Chr4 lin	2464	WIAF   WIAF-57
4	149.40 cR from top of Chr4 lin	1362	WIAF   WIAF-3265
4	149.40 cR from top of Chr4 lin	1364	WIAF   WIAF-3267
4	153 cM	4306	UWGC   125
30	153.60 cR from top of Chr4 lin	3535	WIAF   WIAF-1990
4	174.10 cR from top of Chr4 lin	1479	WIAF   WIAF-3418
4	182.20 cR from top of Chr4 lin	1821	WIAF   WIAF-3760
4	193.30 cR from top of Chr4 lin	1353	WIAF   WIAF-3256
4	193.8 cR from top of Chr4 link	1984	WIAF   WIAF-3923
35	197.2 cR from top of Chr4 link	2913	WIAF   WIAF-812
4	199.00 cR from top of Chr4 lin	3219	WIAF   WIAF-1597
4	199.10 cR from top of Chr4 lin	1618	WIAF   WIAF-3557
4	199.70 cR from top of Chr4 lin	1865	WIAF   WIAF-3804
4	243.0 cR from top of Chr4 link	2739	WIAF   WIAF-452
40	401.1 cR from top of Chr4 link	2152	WIAF   WIAF-482
4	401.1 cR from top of Chr4 link	2153	WIAF   WIAF-483
4	412.2 cR from top of Chr4 link	3031	WIAF   WIAF-931
4	415.9 cR from top of Chr4 link	4564	HU-CHINA   4-197
4	415.9 cR from top of Chr4 link	2568	WIAF   WIAF-197
45	419.4 cR from top of Chr4 link	880	WIAF   WIAF-1116
4	426.7 cR from top of Chr4 link	982	WIAF   WIAF-1408
4	474.6 cR from top of Chr4 link	3019	WIAF   WIAF-919
4	474.6 cR from top of Chr4 link	3020	WIAF   WIAF-920
4	483.0 cR from top of Chr4 link	2712	WIAF   WIAF-407
50	497.5 cR from top of Chr4 link	3981	SHGC/AFFYMETRIX   SNP-SHGC-51763

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	4 499.2 cR from top of Chr4 link	2780	WIAF   WIAF-524
	4 508.3 cR from top of Chr4 link	2815	WIAF   WIAF-610
	4 508.3 cR from top of Chr4 link	2816	WIAF   WIAF-611
	4 522.1 cR from top of Chr4 link	2756	WIAF   WIAF-484
5	4 523.9 cR from top of Chr4 link	2688	WIAF   WIAF-373
	4 526.7 cR from top of Chr4 link	2914	WIAF   WIAF-813
	4 533.1 cR from top of Chr4 link	4181	SHGC/AFFYMETRIX   SNP-SHGC-50672
	4 533.1 cR from top of Chr4 link	785	WIAF   WIAF-2048
	4 538.1 cR from top of Chr4 link	1312	WIAF   WIAF-2201
10	4 543.1 cR from top of Chr4 link	729	WIAF   WIAF-1078
	4 563.3 cR from top of Chr4 link	2179	WIAF   WIAF-561
	4 572.4 cR from top of Chr4 link	3300	WIAF   WIAF-1753
	4 572.9 cR from top of Chr4 link	2344	WIAF   WIAF-2575
	4 602.7 cR from top of Chr4 link	2995	WIAF   WIAF-895
15	4 626.5 cR from top of Chr4 link	2094	WIAF   WIAF-121
	4 626.5 cR from top of Chr4 link	2095	WIAF   WIAF-122
	4 631.4 cR from top of Chr4 link	2364	WIAF   WIAF-2621
	4 631.4 cR from top of Chr4 link	2365	WIAF   WIAF-2623
	4 642.1 cR from top of Chr4 link	2074	WIAF   WIAF-8
20	4 644.6 cR from top of Chr4 link	3823	WIAF   WIAF-2587
	4 644.6 cR from top of Chr4 link	3826	WIAF   WIAF-2591
	4 4p	3986	SHGC/AFFYMETRIX   SNPA-SHGC4-1659
	4 4p	3987	SHGC/AFFYMETRIX   SNPA-SHGC-51324
	4 4p	3991	SHGC/AFFYMETRIX   SNPB-SHGC4-1659
25	4 4p	3992	SHGC/AFFYMETRIX   SNPB-SHGC-51324
	4 4p	3994	SHGC/AFFYMETRIX   SNPC-SHGC-51324
	4 4p	4019	SHGC/AFFYMETRIX   SNP-SHGC4-1525
	4 4p	4200	SHGC/AFFYMETRIX   SNP-SHGC-51310
	4 4p	4201	SHGC/AFFYMETRIX   SNP-SHGC-51312
30	4 4p	4204	SHGC/AFFYMETRIX   SNP-SHGC-51346
	4	4252	MARSHFIELD   MID-7
	4	4119	SHGC/AFFYMETRIX   SNPA-SHGC-14934
	4	4121	SHGC/AFFYMETRIX   SNPA-SHGC-24080
	4	4055	SHGC/AFFYMETRIX   SNPA-SHGC-50187
35	4	4056	SHGC/AFFYMETRIX   SNPA-SHGC-50252
	4	4122	SHGC/AFFYMETRIX   SNPA-SHGC-50922
	4	4057	SHGC/AFFYMETRIX   SNPA-SHGC-50928
	4	4123	SHGC/AFFYMETRIX   SNPA-SHGC-51072
	4	4124	SHGC/AFFYMETRIX   SNPA-SHGC-51160
40	4	4125	SHGC/AFFYMETRIX   SNPA-SHGC-51438
	4	4126	SHGC/AFFYMETRIX   SNPA-SHGC-51690
	4	4129	SHGC/AFFYMETRIX   SNPB-SHGC-14934
	4	4131	SHGC/AFFYMETRIX   SNPB-SHGC-24080
	4	4061	SHGC/AFFYMETRIX   SNPB-SHGC-50187
45	4	4062	SHGC/AFFYMETRIX   SNPB-SHGC-50252
	4	4132	SHGC/AFFYMETRIX   SNPB-SHGC-50922
	4	4063	SHGC/AFFYMETRIX   SNPB-SHGC-50928
	4	4133	SHGC/AFFYMETRIX   SNPB-SHGC-51072
	4	4134	SHGC/AFFYMETRIX   SNPB-SHGC-51160
50	4	4135	SHGC/AFFYMETRIX   SNPB-SHGC-51438

CHROMOSOME	FINE MAP LOCATION	dbSNP	HANDLE   LOCAL
		ASSAY ID	SNP ID
	4	4136	SHGC/AFFYMETRIX   SNPB-SHGC-51690
	4	3958	SHGC/AFFYMETRIX   SNP-SHGC-10699
	4	4005	SHGC/AFFYMETRIX   SNP-SHGC-13008
	4	4150	SHGC/AFFYMETRIX   SNP-SHGC-14139
5	4	4077	SHGC/AFFYMETRIX   SNP-SHGC-16028
	4	4091	SHGC/AFFYMETRIX   SNP-SHGC-17200
	4	4167	SHGC/AFFYMETRIX   SNP-SHGC-23754
	4	4169	SHGC/AFFYMETRIX   SNP-SHGC-24086
	4	4170	SHGC/AFFYMETRIX   SNP-SHGC-24090
10	4	4171	SHGC/AFFYMETRIX   SNP-SHGC-25057
	4	4172	SHGC/AFFYMETRIX   SNP-SHGC-25080
	4	4173	SHGC/AFFYMETRIX   SNP-SHGC-25091
	4	4174	SHGC/AFFYMETRIX   SNP-SHGC-25112
15	4	4175	SHGC/AFFYMETRIX   SNP-SHGC-25184
	4	4017	SHGC/AFFYMETRIX   SNP-SHGC4-1137
	4	4018	SHGC/AFFYMETRIX   SNP-SHGC4-1459
	4	4020	SHGC/AFFYMETRIX   SNP-SHGC4-1597
	4	4021	SHGC/AFFYMETRIX   SNP-SHGC4-1678
	4	4023	SHGC/AFFYMETRIX   SNP-SHGC4-851
20	4	3978	SHGC/AFFYMETRIX   SNP-SHGC-50175
	4	3979	SHGC/AFFYMETRIX   SNP-SHGC-50177
	4	4109	SHGC/AFFYMETRIX   SNP-SHGC-50262
	4	3980	SHGC/AFFYMETRIX   SNP-SHGC-50274
	4	4110	SHGC/AFFYMETRIX   SNP-SHGC-50293
25	4	4176	SHGC/AFFYMETRIX   SNP-SHGC-50311
	4	4177	SHGC/AFFYMETRIX   SNP-SHGC-50320
	4	4111	SHGC/AFFYMETRIX   SNP-SHGC-50369
	4	4024	SHGC/AFFYMETRIX   SNP-SHGC-50475
	4	4179	SHGC/AFFYMETRIX   SNP-SHGC-50477
30	4	4180	SHGC/AFFYMETRIX   SNP-SHGC-50629
	4	4182	SHGC/AFFYMETRIX   SNP-SHGC-50730
	4	4113	SHGC/AFFYMETRIX   SNP-SHGC-50803
	4	4114	SHGC/AFFYMETRIX   SNP-SHGC-50804
	4	4115	SHGC/AFFYMETRIX   SNP-SHGC-50810
35	4	4183	SHGC/AFFYMETRIX   SNP-SHGC-50857
	4	4184	SHGC/AFFYMETRIX   SNP-SHGC-50859
	4	4185	SHGC/AFFYMETRIX   SNP-SHGC-50880
	4	4186	SHGC/AFFYMETRIX   SNP-SHGC-50921
	4	4187	SHGC/AFFYMETRIX   SNP-SHGC-50993
40	4	4025	SHGC/AFFYMETRIX   SNP-SHGC-51011
	4	4188	SHGC/AFFYMETRIX   SNP-SHGC-51034
	4	4189	SHGC/AFFYMETRIX   SNP-SHGC-51046
	4	4190	SHGC/AFFYMETRIX   SNP-SHGC-51122
	4	4191	SHGC/AFFYMETRIX   SNP-SHGC-51140
45	4	4192	SHGC/AFFYMETRIX   SNP-SHGC-51173
	4	4193	SHGC/AFFYMETRIX   SNP-SHGC-51187
	4	4194	SHGC/AFFYMETRIX   SNP-SHGC-51200
	4	4026	SHGC/AFFYMETRIX   SNP-SHGC-51209
	4	4195	SHGC/AFFYMETRIX   SNP-SHGC-51227
50	4	4196	SHGC/AFFYMETRIX   SNP-SHGC-51237

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>		<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
5	4		4197	SHGC/AFFYMETRIX   SNP-SHGC-51240
	4		4198	SHGC/AFFYMETRIX   SNP-SHGC-51242
	4		4199	SHGC/AFFYMETRIX   SNP-SHGC-51249
	4		4202	SHGC/AFFYMETRIX   SNP-SHGC-51323
	4		4203	SHGC/AFFYMETRIX   SNP-SHGC-51340
	4		4205	SHGC/AFFYMETRIX   SNP-SHGC-51387
	4		4027	SHGC/AFFYMETRIX   SNP-SHGC-51411
	4		4028	SHGC/AFFYMETRIX   SNP-SHGC-51435
	4		4029	SHGC/AFFYMETRIX   SNP-SHGC-51467
	4		4206	SHGC/AFFYMETRIX   SNP-SHGC-51477
10	4		4207	SHGC/AFFYMETRIX   SNP-SHGC-51520
	4		4208	SHGC/AFFYMETRIX   SNP-SHGC-51554
	4		4209	SHGC/AFFYMETRIX   SNP-SHGC-51579
	4		4116	SHGC/AFFYMETRIX   SNP-SHGC-51662
	4		4210	SHGC/AFFYMETRIX   SNP-SHGC-51721
15	4		3983	SHGC/AFFYMETRIX   SNP-SHGC-9709
	4		2528	WIAF   WIAF-138
	4		3531	WIAF   WIAF-1986
	4		3686	WIAF   WIAF-2414
	4		3688	WIAF   WIAF-2416
20	4		2950	WIAF   WIAF-849
	4			
25	5	0.00 cR from top of Chr5 linka	3349	WIAF   WIAF-1802
	5	5.2 cR from top of Chr5 linkag	2285	WIAF   WIAF-1331
	5	16.30 cR from top of Chr5 link	3330	WIAF   WIAF-1783
	5	16.30 cR from top of Chr5 link	3331	WIAF   WIAF-1784
	5	18.60 cR from top of Chr5 link	1359	WIAF   WIAF-3262
	5	19.50 cR from top of Chr5 link	1410	WIAF   WIAF-3331
	5	19.70 cR from top of Chr5 link	2013	WIAF   WIAF-1507
	5	36.8 cR from top of Chr5 linka	2953	WIAF   WIAF-852
	5	39.10 cR from top of Chr5 link	1810	WIAF   WIAF-3749
	5	39.10 cR from top of Chr5 link	1813	WIAF   WIAF-3752
30	5	44.5 cR from top of Chr5 linka	3076	WIAF   WIAF-977
	5	45.40 cR from top of Chr5 link	1621	WIAF   WIAF-3560
	5	51.60 cR from top of Chr5 link	1105	WIAF   WIAF-1532
	5	51.60 cR from top of Chr5 link	1415	WIAF   WIAF-3342
	5	57.30 cR from top of Chr5 link	1464	WIAF   WIAF-3399
35	5	62.80 cR from top of Chr5 link	1636	WIAF   WIAF-3575
	5	65.00 cR from top of Chr5 link	3148	WIAF   WIAF-1049
	5	69.40 cR from top of Chr5 link	1986	WIAF   WIAF-3925
	5	69.40 cR from top of Chr5 link	1987	WIAF   WIAF-3926
	5	79.40 cR from top of Chr5 link	3414	WIAF   WIAF-1869
40	5	80.20 cR from top of Chr5 link	1512	WIAF   WIAF-3451
	5	80.30 cR from top of Chr5 link	1665	WIAF   WIAF-3604
	5	82.30 cR from top of Chr5 link	3010	WIAF   WIAF-910
	5	82.80 cR from top of Chr5 link	3249	WIAF   WIAF-1667
	5	82.80 cR from top of Chr5 link	1514	WIAF   WIAF-3453
45	5	84.10 cR from top of Chr5 link	1591	WIAF   WIAF-3530
	5	84.10 cR from top of Chr5 link	1605	WIAF   WIAF-3544
50	5			
	5			

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
5	86.10 cR from top of Chr5 link	3180	WIAF   WIAF-1496
5	87.20 cR from top of Chr5 link	1525	WIAF   WIAF-3464
5	92.30 cR from top of Chr5 link	1608	WIAF   WIAF-3547
5	93.80 cR from top of Chr5 link	1614	WIAF   WIAF-3553
5	97.90 cR from top of Chr5 link	1515	WIAF   WIAF-3454
5	97.90 cR from top of Chr5 link	1517	WIAF   WIAF-3456
5	103.0 cR from top of Chr5 link	2113	WIAF   WIAF-235
5	104.50 cR from top of Chr5 lin	2033	WIAF   WIAF-1594
5	104.50 cR from top of Chr5 lin	3367	WIAF   WIAF-1820
10	104.50 cR from top of Chr5 lin	3368	WIAF   WIAF-1821
5	104.50 cR from top of Chr5 lin	3369	WIAF   WIAF-1822
5	109.00 cR from top of Chr5 lin	3107	WIAF   WIAF-1008
5	117.3 cR from top of Chr5 link	2229	WIAF   WIAF-662
5	117.3 cR from top of Chr5 link	2230	WIAF   WIAF-663
15	121.60 cR from top of Chr5 lin	997	WIAF   WIAF-2053
5	122.30 cR from top of Chr5 lin	1221	WIAF   WIAF-2108
5	122.30 cR from top of Chr5 lin	1222	WIAF   WIAF-2109
5	122.60 cR from top of Chr5 lin	1939	WIAF   WIAF-3878
5	124.3 cR from top of Chr5 link	3145	WIAF   WIAF-1046
20	131.7 cR from top of Chr5 link	3835	WIAF   WIAF-2608
5	131.7 cR from top of Chr5 link	3836	WIAF   WIAF-2609
5	132.90 cR from top of Chr5 lin	1832	WIAF   WIAF-3771
5	140.90 cR from top of Chr5 lin	1215	WIAF   WIAF-2102
5	141.00 cR from top of Chr5 lin	2577	WIAF   WIAF-209
25	141.40 cR from top of Chr5 lin	1467	WIAF   WIAF-3402
5	142.30 cR from top of Chr5 lin	1620	WIAF   WIAF-3559
5	144.10 cR from top of Chr5 lin	3118	WIAF   WIAF-1019
5	153.50 cR from top of Chr5 lin	3282	WIAF   WIAF-1735
5	156.10 cR from top of Chr5 lin	1902	WIAF   WIAF-3841
30	156.40 cR from top of Chr5 lin	1849	WIAF   WIAF-3788
5	163.00 cR from top of Chr5 lin	1272	WIAF   WIAF-2159
5	163.00 cR from top of Chr5 lin	1273	WIAF   WIAF-2160
5	163.00 cR from top of Chr5 lin	1274	WIAF   WIAF-2161
5	169.80 cR from top of Chr5 lin	1788	WIAF   WIAF-3727
35	169.80 cR from top of Chr5 lin	1790	WIAF   WIAF-3729
5	182.0 cR from top of Chr5 link	2478	WIAF   WIAF-74
5	186.40 cR from top of Chr5 lin	691	WIAF   WIAF-1220
5	187.9 cR from top of Chr5 link	3343	WIAF   WIAF-1796
5	194.00 cR from top of Chr5 lin	1918	WIAF   WIAF-3857
40	195.80 cR from top of Chr5 lin	1323	WIAF   WIAF-2213
5	261.7 cR from top of Chr5 link	3269	WIAF   WIAF-1721
5	266.6 cR from top of Chr5 link	2575	WIAF   WIAF-205
5	282.2 cR from top of Chr5 link	2805	WIAF   WIAF-575
5	310.9 cR from top of Chr5 link	2860	WIAF   WIAF-755
45	334.8 cR from top of Chr5 link	2666	WIAF   WIAF-346
5	334.8 cR from top of Chr5 link	2667	WIAF   WIAF-347
5	334.8 cR from top of Chr5 link	2668	WIAF   WIAF-348
5	351.7 cR from top of Chr5 link	2792	WIAF   WIAF-548
5	357.7 cR from top of Chr5 link	2890	WIAF   WIAF-788
50	368.6 cR from top of Chr5 link	3598	WIAF   WIAF-2267

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>		<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
5	368.6 cR from top of Chr5 link	2701	WIAF	WIAF-389
5	372.6 cR from top of Chr5 link	3351	WIAF	WIAF-1804
5	378.7 cR from top of Chr5 link	2627	WIAF	WIAF-287
5	401.5 cR from top of Chr5 link	3523	WIAF	WIAF-1978
5	406.7 cR from top of Chr5 link	3568	WIAF	WIAF-2023
5	425.1 cR from top of Chr5 link	2106	WIAF	WIAF-186
5	425.1 cR from top of Chr5 link	2107	WIAF	WIAF-187
5	431.5 cR from top of Chr5 link	2894	WIAF	WIAF-792
5	431.5 cR from top of Chr5 link	2895	WIAF	WIAF-793
10	437.0 cR from top of Chr5 link	2118	WIAF	WIAF-276
5	441.2 cR from top of Chr5 link	3492	WIAF	WIAF-1947
5	500.0 cR from top of Chr5 link	3374	WIAF	WIAF-1827
5	510.2 cR from top of Chr5 link	1200	WIAF	WIAF-2087
5	532.5 cR from top of Chr5 link	4578	HU-CHINA	5-787
15	532.5 cR from top of Chr5 link	4550	HU-CHINA	5-787-2
5	532.5 cR from top of Chr5 link	2889	WIAF	WIAF-787
5	532.7 cR from top of Chr5 link	1121	WIAF	WIAF-1561
5	534.1 cR from top of Chr5 link	2442	WIAF	WIAF-27
5	537.3 cR from top of Chr5 link	3455	WIAF	WIAF-1910
20	537.4 cR from top of Chr5 link	2677	WIAF	WIAF-359
5	569.8 cR from top of Chr5 link	2211	WIAF	WIAF-631
5		4253	MARSHFIELD	MID-8
5		4052	SHGC/AFFYMETRIX	SNPA-SHGC-16519
25	5	4058	SHGC/AFFYMETRIX	SNPB-SHGC-16519
5		3961	SHGC/AFFYMETRIX	SNP-SHGC-10972
5		4142	SHGC/AFFYMETRIX	SNP-SHGC-13353
5		4160	SHGC/AFFYMETRIX	SNP-SHGC-14742
5		4080	SHGC/AFFYMETRIX	SNP-SHGC-16780
5		4050	SHGC/AFFYMETRIX	SNP-SHGC-9420
30	5	1101	WIAF	WIAF-1520
5		1492	WIAF	WIAF-3431
5		3881	WIAF	WIAF-3942
35	6	0.0 cR from top of Chr6 linkag	3133	WIAF   WIAF-1034
6	1.40 cR from top of Chr6 linka	2028	WIAF	WIAF-1583
6	1.40 cR from top of Chr6 linka	1497	WIAF	WIAF-3436
6	1.40 cR from top of Chr6 linka	1674	WIAF	WIAF-3613
6	1.40 cR from top of Chr6 linka	1782	WIAF	WIAF-3721
40	6	1.40 cR from top of Chr6 linka	1827	WIAF   WIAF-3766
6	1.6 cR from top of Chr6 linkag	2958	WIAF	WIAF-857
6	6.40 cR from top of Chr6 linka	1209	WIAF	WIAF-2096
6	9.80 cR from top of Chr6 linka	1657	WIAF	WIAF-3596
6	9.80 cR from top of Chr6 linka	1658	WIAF	WIAF-3597
45	6	9.80 cR from top of Chr6 linka	1659	WIAF   WIAF-3598
6	17.70 cR from top of Chr6 link	3119	WIAF	WIAF-1020
6	17.80 cR from top of Chr6 link	1373	WIAF	WIAF-3277
6	17.80 cR from top of Chr6 link	1933	WIAF	WIAF-3872
6	17.80 cR from top of Chr6 link	1936	WIAF	WIAF-3875
50	6	20.50 cR from top of Chr6 link	1124	WIAF   WIAF-1567

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
5	6 21.30 cR from top of Chr6 link	1551	WIAF   WIAF-3490
	6 24.50 cR from top of Chr6 link	3066	WIAF   WIAF-967
	6 31.7 cR from top of Chr6 linka	2366	WIAF   WIAF-2627
	6 34.20 cR from top of Chr6 link	1109	WIAF   WIAF-1541
5	6 34.90 cR from top of Chr6 link	3511	WIAF   WIAF-1966
	6 38.00 cR from top of Chr6 link	1733	WIAF   WIAF-3672
	6 41.5 cR from top of Chr6 linka	2522	WIAF   WIAF-131
	6 41.5 cR from top of Chr6 linka	2523	WIAF   WIAF-132
10	6 43.50 cR from top of Chr6 link	3504	WIAF   WIAF-1959
	6 44.20 cR from top of Chr6 link	3211	WIAF   WIAF-1574
	6 46.00 cR from top of Chr6 link	2003	WIAF   WIAF-1460
	6 46.00 cR from top of Chr6 link	2004	WIAF   WIAF-1461
	6 46.00 cR from top of Chr6 link	2005	WIAF   WIAF-1462
15	6 46.60 cR from top of Chr6 link	1116	WIAF   WIAF-1551
	6 46.60 cR from top of Chr6 link	1117	WIAF   WIAF-1552
	6 46.60 cR from top of Chr6 link	1118	WIAF   WIAF-1553
	6 46.60 cR from top of Chr6 link	1119	WIAF   WIAF-1554
	6 46.60 cR from top of Chr6 link	1546	WIAF   WIAF-3485
	6 46.60 cR from top of Chr6 link	1548	WIAF   WIAF-3487
20	6 46.60 cR from top of Chr6 link	1866	WIAF   WIAF-3805
	6 46.60 cR from top of Chr6 link	1992	WIAF   WIAF-3931
	6 46.70 cR from top of Chr6 link	3270	WIAF   WIAF-1722
	6 46.80 cR from top of Chr6 link	1729	WIAF   WIAF-3668
	6 46.80 cR from top of Chr6 link	1732	WIAF   WIAF-3671
25	6 46.80 cR from top of Chr6 link	1735	WIAF   WIAF-3674
	6 46.90 cR from top of Chr6 link	678	WIAF   WIAF-1453
	6 47.00 cR from top of Chr6 link	3260	WIAF   WIAF-1696
	6 47.00 cR from top of Chr6 link	3480	WIAF   WIAF-1935
30	6 47.00 cR from top of Chr6 link	1385	WIAF   WIAF-3290
	6 47.00 cR from top of Chr6 link	1601	WIAF   WIAF-3540
	6 47.00 cR from top of Chr6 link	1905	WIAF   WIAF-3844
	6 47.00 cR from top of Chr6 link	1907	WIAF   WIAF-3846
	6 47.00 cR from top of Chr6 link	2997	WIAF   WIAF-897
	6 47.00 cR from top of Chr6 link	2998	WIAF   WIAF-898
35	6 47.10 cR from top of Chr6 link	1769	WIAF   WIAF-3708
	6 47.20 cR from top of Chr6 link	3108	WIAF   WIAF-1009
	6 47.30 cR from top of Chr6 link	1754	WIAF   WIAF-3693
	6 47.30 cR from top of Chr6 link	1755	WIAF   WIAF-3694
	6 47.30 cR from top of Chr6 link	1757	WIAF   WIAF-3696
40	6 47.70 cR from top of Chr6 link	1219	WIAF   WIAF-2106
	6 47.80 cR from top of Chr6 link	1216	WIAF   WIAF-2103
	6 47.90 cR from top of Chr6 link	1472	WIAF   WIAF-3409
	6 47.90 cR from top of Chr6 link	1474	WIAF   WIAF-3411
	6 50 cM	4317	UWGC   136
45	6 51.1 cR from top of Chr6 linka	3049	WIAF   WIAF-950
	6 52.80 cR from top of Chr6 link	2030	WIAF   WIAF-1586
	6 52.80 cR from top of Chr6 link	2031	WIAF   WIAF-1587
	6 54.6 cR from top of Chr6 linka	3484	WIAF   WIAF-1939
	6 59.5 cR from top of Chr6 linka	2990	WIAF   WIAF-890
50	6 65.50 cR from top of Chr6 link	1188	WIAF   WIAF-2075

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
6	66.60 cR from top of Chr6 link	3220	WIAF   WIAF-1608
6	66.60 cR from top of Chr6 link	3221	WIAF   WIAF-1609
6	69.90 cR from top of Chr6 link	3096	WIAF   WIAF-997
6	71.3 cR from top of Chr6 link	2981	WIAF   WIAF-881
5	79.90 cR from top of Chr6 link	1322	WIAF   WIAF-2212
6	85.50 cR from top of Chr6 link	1850	WIAF   WIAF-3789
6	86.40 cR from top of Chr6 link	1761	WIAF   WIAF-3700
6	89.5 cR from top of Chr6 link	2510	WIAF   WIAF-116
6	89.60 cR from top of Chr6 link	3022	WIAF   WIAF-922
10	90.50 cR from top of Chr6 link	1528	WIAF   WIAF-3467
6	90.50 cR from top of Chr6 link	1532	WIAF   WIAF-3471
6	93.60 cR from top of Chr6 link	3172	WIAF   WIAF-1486
6	95.50 cR from top of Chr6 link	3251	WIAF   WIAF-1669
6	95.50 cR from top of Chr6 link	3252	WIAF   WIAF-1670
15	95.50 cR from top of Chr6 link	1818	WIAF   WIAF-3757
6	96.00 cR from top of Chr6 link	1807	WIAF   WIAF-3746
6	99.8 cR from top of Chr6 link	2090	WIAF   WIAF-105
6	99.8 cR from top of Chr6 link	2091	WIAF   WIAF-106
6	102.40 cR from top of Chr6 lin	3244	WIAF   WIAF-1654
20	105.40 cR from top of Chr6 lin	1783	WIAF   WIAF-3722
6	106.40 cR from top of Chr6 lin	3167	WIAF   WIAF-1476
6	106.90 cR from top of Chr6 lin	1417	WIAF   WIAF-3344
6	116.20 cR from top of Chr6 lin	1809	WIAF   WIAF-3748
6	116.60 cR from top of Chr6 lin	3420	WIAF   WIAF-1875
25	116.60 cR from top of Chr6 lin	3421	WIAF   WIAF-1876
6	116.60 cR from top of Chr6 lin	3422	WIAF   WIAF-1877
6	116.60 cR from top of Chr6 lin	3423	WIAF   WIAF-1878
6	116.60 cR from top of Chr6 lin	3424	WIAF   WIAF-1879
6	116.60 cR from top of Chr6 lin	3425	WIAF   WIAF-1880
30	116.60 cR from top of Chr6 lin	3426	WIAF   WIAF-1881
6	116.60 cR from top of Chr6 lin	1329	WIAF   WIAF-2219
6	116.90 cR from top of Chr6 lin	1239	WIAF   WIAF-2126
6	117.00 cR from top of Chr6 lin	1582	WIAF   WIAF-3521
6	124.20 cR from top of Chr6 lin	1284	WIAF   WIAF-2171
35	125.4 cR from top of Chr6 link	3441	WIAF   WIAF-1896
6	125.4 cR from top of Chr6 link	3828	WIAF   WIAF-2597
6	125.4 cR from top of Chr6 link	2716	WIAF   WIAF-415
6	125.40 cR from top of Chr6 lin	1592	WIAF   WIAF-3531
6	125.80 cR from top of Chr6 lin	3254	WIAF   WIAF-1674
40	131.20 cR from top of Chr6 lin	1535	WIAF   WIAF-3474
6	137.90 cR from top of Chr6 lin	1706	WIAF   WIAF-3645
6	144.50 cR from top of Chr6 lin	1141	WIAF   WIAF-1604
6	144.50 cR from top of Chr6 lin	1860	WIAF   WIAF-3799
6	144.90 cR from top of Chr6 lin	3418	WIAF   WIAF-1873
45	145.60 cR from top of Chr6 lin	3579	WIAF   WIAF-2034
6	147.30 cR from top of Chr6 lin	1771	WIAF   WIAF-3710
6	150.40 cR from top of Chr6 lin	1150	WIAF   WIAF-1623
6	150.40 cR from top of Chr6 lin	1176	WIAF   WIAF-1701
6	154.90 cR from top of Chr6 lin	1563	WIAF   WIAF-3502
50	155.70 cR from top of Chr6 lin	1737	WIAF   WIAF-3676

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
6	155.70 cR from top of Chr6 lin 1738	WIAF	WIAF-3677
6	159.00 cR from top of Chr6 lin 1867	WIAF	WIAF-3806
6	165.70 cR from top of Chr6 lin 3499	WIAF	WIAF-1954
6	165.70 cR from top of Chr6 lin 3500	WIAF	WIAF-1955
5	6 166.40 cR from top of Chr6 lin 1465	WIAF	WIAF-3400
	6 166.80 cR from top of Chr6 lin 1513	WIAF	WIAF-3452
	6 175.90 cR from top of Chr6 lin 3015	WIAF	WIAF-915
	6 177.0 cR from top of Chr6 link 2798	WIAF	WIAF-556
	6 177.0 cR from top of Chr6 link 2799	WIAF	WIAF-557
10	6 177.0 cR from top of Chr6 link 2800	WIAF	WIAF-558
	6 178.40 cR from top of Chr6 lin 1896	WIAF	WIAF-3835
	6 180.3 cR from top of Chr6 link 2611	WIAF	WIAF-257
	6 180.3 cR from top of Chr6 link 2612	WIAF	WIAF-258
	6 180.4 cR from top of Chr6 link 951	WIAF	WIAF-1335
15	6 180.4 cR from top of Chr6 link 952	WIAF	WIAF-1336
	6 180.4 cR from top of Chr6 link 953	WIAF	WIAF-1337
	6 180.4 cR from top of Chr6 link 954	WIAF	WIAF-1338
	6 180.4 cR from top of Chr6 link 955	WIAF	WIAF-1339
	6 180.4 cR from top of Chr6 link 956	WIAF	WIAF-1340
20	6 180.4 cR from top of Chr6 link 957	WIAF	WIAF-1341
	6 180.4 cR from top of Chr6 link 958	WIAF	WIAF-1342
	6 180.4 cR from top of Chr6 link 1038	WIAF	WIAF-4120
	6 184.80 cR from top of Chr6 lin 1426	WIAF	WIAF-3354
	6 187.1 cR from top of Chr6 link 3565	WIAF	WIAF-2020
25	6 187.1 cR from top of Chr6 link 3566	WIAF	WIAF-2021
	6 187.7 cR from top of Chr6 link 1993	WIAF	WIAF-3932
	6 187.7 cR from top of Chr6 link -1996	WIAF	WIAF-3935
	6 187.8 cR from top of Chr6 link 2529	WIAF	WIAF-139
	6 188.2 cR from top of Chr6 link 1660	WIAF	WIAF-3599
30	6 188.40 cR from top of Chr6 lin 1427	WIAF	WIAF-3355
	6 189.00 cR from top of Chr6 lin 1299	WIAF	WIAF-2186
	6 190.30 cR from top of Chr6 lin 1440	WIAF	WIAF-3369
	6 190.30 cR from top of Chr6 lin 1442	WIAF	WIAF-3371
	6 190.30 cR from top of Chr6 lin 1443	WIAF	WIAF-3373
35	6 201.1 cR from top of Chr6 link 1290	WIAF	WIAF-2177
	6 201.10 cR from top of Chr6 lin 1763	WIAF	WIAF-3702
	6 201.10 cR from top of Chr6 lin 1765	WIAF	WIAF-3704
	6 203.9 cR from top of Chr6 link 2505	WIAF	WIAF-110
	6 212.4 cR from top of Chr6 link 3105	WIAF	WIAF-1006
40	6 212.6 cR from top of Chr6 link 3837	WIAF	WIAF-2610
	6 217.6 cR from top of Chr6 link 3016	WIAF	WIAF-916
	6 218.7 cR from top of Chr6 link 3293	WIAF	WIAF-1746
	6 249.6 cR from top of Chr6 link 857	WIAF	WIAF-1060
	6 249.6 cR from top of Chr6 link 1063	WIAF	WIAF-4188
45	6 256.2 cR from top of Chr6 link 1845	WIAF	WIAF-3784
	6 275.6 cR from top of Chr6 link 2499	WIAF	WIAF-98
	6 276.6 cR from top of Chr6 link 3327	WIAF	WIAF-1780
	6 331.1 cR from top of Chr6 link 2907	WIAF	WIAF-805
	6 487.0 cR from top of Chr6 link 2809	WIAF	WIAF-589
50	6 576.9 cR from top of Chr6 link 2296	WIAF	WIAF-2281

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
6	625.0 cR from top of Chr6 link	2084	WIAF   WIAF-78
6	706.1 cR from top of Chr6 link	2945	WIAF   WIAF-844
6	706.1 cR from top of Chr6 link	2946	WIAF   WIAF-845
6	711.4 cR from top of Chr6 link	2808	WIAF   WIAF-584
5	729.2 cR from top of Chr6 link	4588	HU-CHINA   6-985
6	729.2 cR from top of Chr6 link	3084	WIAF   WIAF-985
6	734.7 cR from top of Chr6 link	2274	WIAF   WIAF-736
6	734.7 cR from top of Chr6 link	2275	WIAF   WIAF-737
6	736.4 cR from top of Chr6 link	1784	WIAF   WIAF-3723
10	739.6 cR from top of Chr6 link	2158	WIAF   WIAF-492
6	799.2 cR from top of Chr6 link	2073	WIAF   WIAF-3
6	812.0 cR from top of Chr6 link	2747	WIAF   WIAF-466
6	822.5 cR from top of Chr6 link	2193	WIAF   WIAF-592
6	837.8 cR from top of Chr6 link	3081	WIAF   WIAF-982
15	846.2 cR from top of Chr6 link	2386	WIAF   WIAF-2680
6	856.6 cR from top of Chr6 link	2498	WIAF   WIAF-97
6	858.4 cR from top of Chr6 link	2080	WIAF   WIAF-30
6	860.5 cR from top of Chr6 link	2865	WIAF   WIAF-762
6		4218	MARSHFIELD   MID-10
20	6	4219	MARSHFIELD   MID-11
6		4254	MARSHFIELD   MID-9
6		4117	SHGC/AFFYMETRIX   SNPA-SHGC-13699
6		3988	SHGC/AFFYMETRIX   SNPA-SHGC-6809
6		4127	SHGC/AFFYMETRIX   SNPB-SHGC-13699
25	6	3993	SHGC/AFFYMETRIX   SNPB-SHGC-6809
6		3960	SHGC/AFFYMETRIX   SNP-SHGC-10969
6		4002	SHGC/AFFYMETRIX   SNP-SHGC-12214
6		4149	SHGC/AFFYMETRIX   SNP-SHGC-14111
6		4152	SHGC/AFFYMETRIX   SNP-SHGC-14233
30	6	4158	SHGC/AFFYMETRIX   SNP-SHGC-14719
6		3975	SHGC/AFFYMETRIX   SNP-SHGC-34704
6		3977	SHGC/AFFYMETRIX   SNP-SHGC-44682
6		4042	SHGC/AFFYMETRIX   SNP-SHGC-8858
6		3149	WIAF   WIAF-1050
35	6	1107	WIAF   WIAF-1539
6		3257	WIAF   WIAF-1685
6		3877	WIAF   WIAF-2678
6		1505	WIAF   WIAF-3444
6		1545	WIAF   WIAF-3484
40	6	1616	WIAF   WIAF-3555
6		1781	WIAF   WIAF-3720
6		1787	WIAF   WIAF-3726
6		1789	WIAF   WIAF-3728
6		1791	WIAF   WIAF-3730
45	6	1793	WIAF   WIAF-3732
6		1795	WIAF   WIAF-3734
6		1801	WIAF   WIAF-3740
6		1851	WIAF   WIAF-3790
6		1904	WIAF   WIAF-3843
50	6	1932	WIAF   WIAF-3871

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
6		1935	WIAF   WIAF-3874
6		1938	WIAF   WIAF-3877
6		2972	WIAF   WIAF-872
6		2973	WIAF   WIAF-873
5	6	3063	WIAF   WIAF-964
	6	3086	WIAF   WIAF-987
10	7 2.20 cR from top of Chr7 link a	1804	WIAF   WIAF-3743
	7 5.20 cR from top of Chr7 link a	1300	WIAF   WIAF-2189
	7 18.10 cR from top of Chr7 link	1759	WIAF   WIAF-3698
	7 19.00 cR from top of Chr7 link	1457	WIAF   WIAF-3390
	7 22.00 cR from top of Chr7 link	1913	WIAF   WIAF-3852
15	7 26.4 cR from top of Chr7 link a	2926	WIAF   WIAF-825
	7 29.10 cR from top of Chr7 link	1717	WIAF   WIAF-3656
	7 29.10 cR from top of Chr7 link	1796	WIAF   WIAF-3735
	7 29.10 cR from top of Chr7 link	1808	WIAF   WIAF-3747
	7 34.8 cR from top of Chr7 link a	3832	WIAF   WIAF-2601
	7 37.3 cR from top of Chr7 link a	4579	HU-CHINA   7-349
20	7 37.3 cR from top of Chr7 link a	4551	HU-CHINA   7-349-2
	7 37.3 cR from top of Chr7 link a	2669	WIAF   WIAF-349
	7 37.3 cR from top of Chr7 link a	2670	WIAF   WIAF-350
	7 39.90 cR from top of Chr7 link	1493	WIAF   WIAF-3432
	7 50.00 cR from top of Chr7 link	1526	WIAF   WIAF-3465
25	7 58.9 cR from top of Chr7 link a	774	WIAF   WIAF-1405
	7 64.6 cR from top of Chr7 link a	3807	WIAF   WIAF-2559
	7 70.70 cR from top of Chr7 link	2928	WIAF   WIAF-827
	7 70.70 cR from top of Chr7 link	2929	WIAF   WIAF-828
	7 71.50 cR from top of Chr7 link	1220	WIAF   WIAF-2107
30	7 77.10 cR from top of Chr7 link	1291	WIAF   WIAF-2178
	7 77.10 cR from top of Chr7 link	1292	WIAF   WIAF-2179
	7 83.60 cR from top of Chr7 link	1401	WIAF   WIAF-3321
	7 89.0 cR from top of Chr7 link a	2616	WIAF   WIAF-263
	7 90.20 cR from top of Chr7 link	2042	WIAF   WIAF-1631
35	7 93.2 cR from top of Chr7 link a	2771	WIAF   WIAF-514
	7 93.90 cR from top of Chr7 link	1949	WIAF   WIAF-3888
	7 98.00 cR from top of Chr7 link	1768	WIAF   WIAF-3707
	7 102.30 cR from top of Chr7 lin	1664	WIAF   WIAF-3603
	7 105.20 cR from top of Chr7 lin	668	WIAF   WIAF-1240
40	7 105.20 cR from top of Chr7 lin	669	WIAF   WIAF-1332
	7 106 cM	4324	UWGC   143
	7 109.50 cR from top of Chr7 lin	4573	HU-CHINA   7-1100
	7 109.50 cR from top of Chr7 lin	667	WIAF   WIAF-1100
	7 109.90 cR from top of Chr7 lin	4572	HU-CHINA   7-1495
45	7 109.90 cR from top of Chr7 lin	3179	WIAF   WIAF-1495
	7 110.9 cR from top of Chr7 link	3375	WIAF   WIAF-1828
	7 110.9 cR from top of Chr7 link	3376	WIAF   WIAF-1829
	7 111.60 cR from top of Chr7 lin	3168	WIAF   WIAF-1477
	7 111.60 cR from top of Chr7 lin	3169	WIAF   WIAF-1478
50	7 112.00 cR from top of Chr7 lin	3563	WIAF   WIAF-2018

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	7 112.00 cR from top of Chr7 lin 1815	WIAF	WIAF-3754
	7 112.00 cR from top of Chr7 lin 1816	WIAF	WIAF-3755
	7 112.30 cR from top of Chr7 lin 4574	HU-CHINA	7-1510
	7 112.30 cR from top of Chr7 lin 1098	WIAF	WIAF-1510
5	7 112.90 cR from top of Chr7 lin 1199	WIAF	WIAF-2086
	7 113.40 cR from top of Chr7 lin 1927	WIAF	WIAF-3866
	7 117.20 cR from top of Chr7 lin 4593	HU-CHINA	7-1680
	7 117.20 cR from top of Chr7 lin 4594	HU-CHINA	7-1680-2
	7 117.20 cR from top of Chr7 lin 4595	HU-CHINA	7-1680-3
10	7 117.20 cR from top of Chr7 lin 1167	WIAF	WIAF-1679
	7 117.20 cR from top of Chr7 lin 1168	WIAF	WIAF-1680
	7 117.20 cR from top of Chr7 lin 1169	WIAF	WIAF-1681
	7 119.80 cR from top of Chr7 lin 1180	WIAF	WIAF-1710
	7 122.6 cR from top of Chr7 link 2546	WIAF	WIAF-167
15	7 125.50 cR from top of Chr7 lin 1381	WIAF	WIAF-3285
	7 126.60 cR from top of Chr7 lin 1980	WIAF	WIAF-3919
	7 126.60 cR from top of Chr7 lin 1981	WIAF	WIAF-3920
	7 129.10 cR from top of Chr7 lin 1387	WIAF	WIAF-3292
	7 129.90 cR from top of Chr7 lin 1711	WIAF	WIAF-3650
20	7 135.3 cR from top of Chr7 link 3002	WIAF	WIAF-902
	7 136.50 cR from top of Chr7 lin 3861	WIAF	WIAF-2651
	7 139.70 cR from top of Chr7 lin 1839	WIAF	WIAF-3778
	7 147.7 cR from top of Chr7 link 3357	WIAF	WIAF-1810
	7 150.1 cR from top of Chr7 link 3571	WIAF	WIAF-2026
25	7 150.1 cR from top of Chr7 link 3572	WIAF	WIAF-2027
	7 155.00 cR from top of Chr7 lin 711	WIAF	WIAF-1447
	7 155.00 cR from top of Chr7 lin 712	WIAF	WIAF-1448
	7 165.60 cR from top of Chr7 lin 1777	WIAF	WIAF-3716
	7 165.60 cR from top of Chr7 lin 1778	WIAF	WIAF-3717
30	7 169.00 cR from top of Chr7 lin 1136	WIAF	WIAF-1599
	7 172.90 cR from top of Chr7 lin 3227	WIAF	WIAF-1627
	7 176.60 cR from top of Chr7 lin 1743	WIAF	WIAF-3682
	7 182.40 cR from top of Chr7 lin 2039	WIAF	WIAF-1620
	7 182.40 cR from top of Chr7 lin 1298	WIAF	WIAF-2185
35	7 183.2 cR from top of Chr7 link 3505	WIAF	WIAF-1960
	7 184.00 cR from top of Chr7 lin 1727	WIAF	WIAF-3666
	7 184.00 cR from top of Chr7 lin 2901	WIAF	WIAF-799
	7 187.2 cR from top of Chr7 link 2214	WIAF	WIAF-636
	7 390.2 cR from top of Chr7 link 2603	WIAF	WIAF-247
40	7 399.5 cR from top of Chr7 link 2215	WIAF	WIAF-637
	7 446.9 cR from top of Chr7 link 3370	WIAF	WIAF-1823
	7 453.2 cR from top of Chr7 link 3060	WIAF	WIAF-961
	7 456.7 cR from top of Chr7 link 2908	WIAF	WIAF-806
	7 467.5 cR from top of Chr7 link 2769	WIAF	WIAF-509
45	7 467.6 cR from top of Chr7 link 2828	WIAF	WIAF-644
	7 476.3 cR from top of Chr7 link 4580	HU-CHINA	7-1773
	7 476.3 cR from top of Chr7 link 4552	HU-CHINA	7-1773-2
	7 476.3 cR from top of Chr7 link 2502	WIAF	WIAF-104
	7 476.3 cR from top of Chr7 link 3319	WIAF	WIAF-1772
50	7 476.3 cR from top of Chr7 link 3320	WIAF	WIAF-1773

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	7 479.2 cR from top of Chr7 link	2187	WIAF   WIAF-570
	7 491.0 cR from top of Chr7 link	4565	HU-CHINA   7-1781
	7 491.0 cR from top of Chr7 link	3328	WIAF   WIAF-1781
	7 493.0 cR from top of Chr7 link	2354	WIAF   WIAF-2594
5	7 493.0 cR from top of Chr7 link	2355	WIAF   WIAF-2595
	7 495.4 cR from top of Chr7 link	867	WIAF   WIAF-1080
	7 495.4 cR from top of Chr7 link	1265	WIAF   WIAF-2152
	7 495.4 cR from top of Chr7 link	1058	WIAF   WIAF-4174
	7 497.2 cR from top of Chr7 link	2548	WIAF   WIAF-169
10	7 502.4 cR from top of Chr7 link	2909	WIAF   WIAF-807
	7 514.8 cR from top of Chr7 link	2487	WIAF   WIAF-85
	7 522.1 cR from top of Chr7 link	3329	WIAF   WIAF-1782
	7 530.0 cR from top of Chr7 link	2866	WIAF   WIAF-764
	7 568.6 cR from top of Chr7 link	2678	WIAF   WIAF-360
15	7 568.6 cR from top of Chr7 link	853	WIAF   WIAF-361
	7 598.6 cR from top of Chr7 link	3338	WIAF   WIAF-1791
	7 602.0 cR from top of Chr7 link	2534	WIAF   WIAF-147
	7 602.0 cR from top of Chr7 link	2535	WIAF   WIAF-148
	7 603.1 cR from top of Chr7 link	3284	WIAF   WIAF-1737
20	7 603.1 cR from top of Chr7 link	3285	WIAF   WIAF-1738
	7 621.5 cR from top of Chr7 link	730	WIAF   WIAF-1087
	7 646.3 cR from top of Chr7 link	3154	WIAF   WIAF-1055
	7 663.1 cR from top of Chr7 link	2444	WIAF   WIAF-32
	7 663.1 cR from top of Chr7 link	2445	WIAF   WIAF-33
25	7 668.3 cR from top of Chr7 link	3332	WIAF   WIAF-1785
	7 669.9 cR from top of Chr7 link	3536	WIAF   WIAF-1991
	7 670.2 cR from top of Chr7 link	2751	WIAF   WIAF-473
	7 670.6 cR from top of Chr7 link	3522	WIAF   WIAF-1977
	7 333 EXAMPLE   CTFR-tttdel		
30	7 3954	MARSHFIELD	MID-1
	7 3955	MARSHFIELD	MID-2
	7 3956	MARSHFIELD	MID-3
	7 4247	MARSHFIELD	MID-4
	7 4250	MARSHFIELD	MID-5
35	7 4251	MARSHFIELD	MID-6
	7 4144	SHGC/AFFYMETRIX	SNP-SHGC-13664
	7 4084	SHGC/AFFYMETRIX	SNP-SHGC-16934
	7 4090	SHGC/AFFYMETRIX	SNP-SHGC-17167
	7 4100	SHGC/AFFYMETRIX	SNP-SHGC-19036
40	7 3973	SHGC/AFFYMETRIX	SNP-SHGC-32515
	7 3195	WIAF	WIAF-1530
	7 1132	WIAF	WIAF-1579
	7 2559	WIAF	WIAF-183
	7 1264	WIAF	WIAF-2151
45	7 1688	WIAF	WIAF-3627
	7 2425	WIAF	WIAF-5
	7 2840	WIAF	WIAF-678
	7 3082	WIAF	WIAF-983

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
8	0.1 cR from top of Chr8 linkag	2267	WIAF   WIAF-724
8	0.1 cR from top of Chr8 linkag	2268	WIAF   WIAF-725
8	0.70 cR from top of Chr8 linka	1785	WIAF   WIAF-3724
8	6.50 cR from top of Chr8 linka	3442	WIAF   WIAF-1897
5	6.50 cR from top of Chr8 linka	3443	WIAF   WIAF-1898
8	8.20 cR from top of Chr8 linka	1895	WIAF   WIAF-3834
8	11.1 cR from top of Chr8 linka	3840	WIAF   WIAF-2614
8	13.40 cR from top of Chr8 link	1281	WIAF   WIAF-2168
8	13.40 cR from top of Chr8 link	1282	WIAF   WIAF-2169
10	15.50 cR from top of Chr8 link	1418	WIAF   WIAF-3345
8	15.50 cR from top of Chr8 link	1419	WIAF   WIAF-3346
8	20.40 cR from top of Chr8 link	3537	WIAF   WIAF-1992
8	20.40 cR from top of Chr8 link	3538	WIAF   WIAF-1993
8	22.7 cR from top of Chr8 linka	2624	WIAF   WIAF-283
15	30.70 cR from top of Chr8 link	2053	WIAF   WIAF-1709
8	31.9 cR from top of Chr8 linka	3476	WIAF   WIAF-1931
8	33.2 cR from top of Chr8 linka	2613	WIAF   WIAF-259
8	33.2 cR from top of Chr8 linka	2614	WIAF   WIAF-260
8	37.0 cR from top of Chr8 linka	2476	WIAF   WIAF-72
20	39.90 cR from top of Chr8 link	1233	WIAF   WIAF-2120
8	40.90 cR from top of Chr8 link	3218	WIAF   WIAF-1596
8	42.70 cR from top of Chr8 link	1100	WIAF   WIAF-1517
8	43.70 cR from top of Chr8 link	1149	WIAF   WIAF-1622
8	43.90 cR from top of Chr8 link	1894	WIAF   WIAF-3833
25	44.40 cR from top of Chr8 link	1481	WIAF   WIAF-3420
8	47.90 cR from top of Chr8 link	1857	WIAF   WIAF-3796
8	55.4 cR from top of Chr8 linka	2457	WIAF   WIAF-48
8	55.4 cR from top of Chr8 linka	2458	WIAF   WIAF-49
8	60.1 cR from top of Chr8 linka	1040	WIAF   WIAF-4128
30	62.60 cR from top of Chr8 link	1174	WIAF   WIAF-1693
8	62.70 cR from top of Chr8 link	3275	WIAF   WIAF-1728
8	62.80 cR from top of Chr8 link	1870	WIAF   WIAF-3809
8	63.30 cR from top of Chr8 link	1682	WIAF   WIAF-3621
8	68.70 cR from top of Chr8 link	1843	WIAF   WIAF-3782
35	80.90 cR from top of Chr8 link	2024	WIAF   WIAF-1565
8	81.50 cR from top of Chr8 link	1133	WIAF   WIAF-1580
8	81.50 cR from top of Chr8 link	3302	WIAF   WIAF-1755
8	88.30 cR from top of Chr8 link	1197	WIAF   WIAF-2084
8	95.3 cR from top of Chr8 linka	2674	WIAF   WIAF-356
40	95.3 cR from top of Chr8 linka	2675	WIAF   WIAF-357
8	96.20 cR from top of Chr8 link	1971	WIAF   WIAF-3910
8	96.3 cR from top of Chr8 linka	3092	WIAF   WIAF-993
8	98.0 cR from top of Chr8 linka	2920	WIAF   WIAF-819
8	101.00 cR from top of Chr8 lin	708	WIAF   WIAF-1406
45	101.3 cR from top of Chr8 link	2967	WIAF   WIAF-867
8	103.7 cR from top of Chr8 link	2933	WIAF   WIAF-832
8	105.80 cR from top of Chr8 lin	1205	WIAF   WIAF-2092
8	108.90 cR from top of Chr8 lin	1739	WIAF   WIAF-3678
8	109.00 cR from top of Chr8 lin	1163	WIAF   WIAF-1666
50	109.80 cR from top of Chr8 lin	1713	WIAF   WIAF-3652

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
8	113.70 cR from top of Chr8 lin 2029	WIAF   WIAF-1584	
8	113.70 cR from top of Chr8 lin 1336	WIAF   WIAF-2226	
8	115.8 cR from top of Chr8 link 2262	WIAF   WIAF-716	
8	115.8 cR from top of Chr8 link 2263	WIAF   WIAF-717	
5	8 118.30 cR from top of Chr8 lin 1350	WIAF   WIAF-3253	
	8 118.30 cR from top of Chr8 lin 1352	WIAF   WIAF-3255	
	8 118.8 cR from top of Chr8 link 2722	WIAF   WIAF-426	
	8 118.8 cR from top of Chr8 link 2723	WIAF   WIAF-427	
	8 119.00 cR from top of Chr8 lin 1447	WIAF   WIAF-3377	
10	8 119.00 cR from top of Chr8 lin 1448	WIAF   WIAF-3378	
	8 121.4 cR from top of Chr8 link 3629	WIAF   WIAF-2357	
	8 124.1 cR from top of Chr8 link 2686	WIAF   WIAF-369	
	8 124.1 cR from top of Chr8 link 2687	WIAF   WIAF-370	
	8 126.30 cR from top of Chr8 lin 1863	WIAF   WIAF-3802	
15	8 126.30 cR from top of Chr8 lin 1864	WIAF   WIAF-3803	
	8 126.50 cR from top of Chr8 lin 1213	WIAF   WIAF-2100	
	8 126.50 cR from top of Chr8 lin 1234	WIAF   WIAF-2121	
	8 126.60 cR from top of Chr8 lin 1326	WIAF   WIAF-2216	
	8 132.00 cR from top of Chr8 lin 1672	WIAF   WIAF-361	
20	8 133.90 cR from top of Chr8 lin 1742	WIAF   WIAF-3681	
	8 147.10 cR from top of Chr8 lin 3192	WIAF   WIAF-1516	
	8 164.20 cR from top of Chr8 lin 3128	WIAF   WIAF-1029	
	8 164.70 cR from top of Chr8 lin 3546	WIAF   WIAF-2001	
	8 166.40 cR from top of Chr8 lin 3240	WIAF   WIAF-1650	
25	8 166.40 cR from top of Chr8 lin 3241	WIAF   WIAF-1651	
	8 166.40 cR from top of Chr8 lin 1847	WIAF   WIAF-3786	
	8 166.40 cR from top of Chr8 lin 3009	WIAF   WIAF-909	
	8 233.1 cR from top of Chr8 link 3639	WIAF   WIAF-2367	
	8 410.4 cR from top of Chr8 link 3028	WIAF   WIAF-928	
30	8 416.4 cR from top of Chr8 link 2474	WIAF   WIAF-69	
	8 416.4 cR from top of Chr8 link 2475	WIAF   WIAF-70	
	8 435.6 cR from top of Chr8 link 2133	WIAF   WIAF-354	
	8 435.6 cR from top of Chr8 link 2134	WIAF   WIAF-355	
	8 441.8 cR from top of Chr8 link 3430	WIAF   WIAF-1885	
35	8 466.7 cR from top of Chr8 link 3391	WIAF   WIAF-1846	
	8 514.9 cR from top of Chr8 link 2497	WIAF   WIAF-96	
	8 541.5 cR from top of Chr8 link 3392	WIAF   WIAF-1847	
	8 579.6 cR from top of Chr8 link 3873	WIAF   WIAF-2672	
	8 588.3 cR from top of Chr8 link 3413	WIAF   WIAF-1868	
40	8 591.7 cR from top of Chr8 link 3526	WIAF   WIAF-1981	
	8 592.1 cR from top of Chr8 link 2157	WIAF   WIAF-490	
	8 592.1 cR from top of Chr8 link 829	WIAF   WIAF-491	
	8 592.4 cR from top of Chr8 link 762	WIAF   WIAF-1351	
	8 625.1 cR from top of Chr8 link 2459	WIAF   WIAF-50	
45	8 625.4 cR from top of Chr8 link 2664	WIAF   WIAF-341	
	8 628.6 cR from top of Chr8 link 2202	WIAF   WIAF-615	
	8 653.0 cR from top of Chr8 link 2864	WIAF   WIAF-761	
	8 656.3 cR from top of Chr8 link 934	WIAF   WIAF-1291	
	8 659.2 cR from top of Chr8 link 3088	WIAF   WIAF-989	
50	8 670.0 cR from top of Chr8 link 2807	WIAF   WIAF-583	

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
5	8 681.6 cR from top of Chr8 link	2839	WIAF   WIAF-677
	8 689.6 cR from top of Chr8 link	775	WIAF   WIAF-1410
	8 708.8 cR from top of Chr8 link	3126	WIAF   WIAF-1027
	8 717.8 cR from top of Chr8 link	2504	WIAF   WIAF-108
	8 731.6 cR from top of Chr8 link	2239	WIAF   WIAF-676
	8 791.3 cR from top of Chr8 link	742	WIAF   WIAF-1186
	8	4220	MARSHFIELD   MID-12
	8	4000	SHGC/AFFYMETRIX   SNP-SHGC-12093
	8	4140	SHGC/AFFYMETRIX   SNP-SHGC-13126
	10 8	4065	SHGC/AFFYMETRIX   SNP-SHGC-13448
15	8	3984	SHGC/AFFYMETRIX   SNP-SHGC-9711
	8	3143	WIAF   WIAF-1044
	8	3171	WIAF   WIAF-1482
	8	3292	WIAF   WIAF-1745
	8	3347	WIAF   WIAF-1800
	8	3447	WIAF   WIAF-1902
	8	3700	WIAF   WIAF-2428
	8	3779	WIAF   WIAF-2507
	8	1634	WIAF   WIAF-3573
	20 8	3890	WIAF   WIAF-3951
25	8	3908	WIAF   WIAF-3985
	8	2742	WIAF   WIAF-457
	8	2743	WIAF   WIAF-458
	8	2488	WIAF   WIAF-86
	8	2965	WIAF   WIAF-865
	8	3095	WIAF   WIAF-996
	9 0.00 cR from top of Chr9 linka	1358	WIAF   WIAF-3261
	9 0.00 cR from top of Chr9 linka	1393	WIAF   WIAF-3298
	9 7.40 cR from top of Chr9 linka	1942	WIAF   WIAF-3881
	9 12.10 cR from top of Chr9 link	1400	WIAF   WIAF-3320
30	9 13.00 cR from top of Chr9 link	1155	WIAF   WIAF-1640
	9 13.00 cR from top of Chr9 link	1156	WIAF   WIAF-1641
	9 15.60 cR from top of Chr9 link	2027	WIAF   WIAF-1582
	9 15.60 cR from top of Chr9 link	1661	WIAF   WIAF-3600
	9 19.60 cR from top of Chr9 link	1279	WIAF   WIAF-2166
	9 25.00 cR from top of Chr9 link	688	WIAF   WIAF-1194
	9 28.90 cR from top of Chr9 link	1749	WIAF   WIAF-3688
	9 29.5 cR from top of Chr9 linka	2105	WIAF   WIAF-185
	9 30.0 cR from top of Chr9 linka	2777	WIAF   WIAF-521
	9 30.0 cR from top of Chr9 linka	3056	WIAF   WIAF-957
35	9 44.00 cR from top of Chr9 link	3464	WIAF   WIAF-1919
	9 55.6 cR from top of Chr9 linka	3298	WIAF   WIAF-1751
	9 55.6 cR from top of Chr9 linka	3299	WIAF   WIAF-1752
	9 57.40 cR from top of Chr9 link	1283	WIAF   WIAF-2170
	9 57.50 cR from top of Chr9 link	1403	WIAF   WIAF-3323
	9 57.80 cR from top of Chr9 link	2556	WIAF   WIAF-180
	9 62.0 cR from top of Chr9 linka	4575	HU-CHINA   9-870
	9 62.0 cR from top of Chr9 linka	2970	WIAF   WIAF-870

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
9	62.70 cR from top of Chr9 link	3246	WIAF   WIAF-1657
9	62.70 cR from top of Chr9 link	3247	WIAF   WIAF-1658
9	64.0 cR from top of Chr9 link	2272	WIAF   WIAF-731
9	64.10 cR from top of Chr9 link	2043	WIAF   WIAF-1634
5	65.20 cR from top of Chr9 link	1148	WIAF   WIAF-1619
9	67.40 cR from top of Chr9 link	1318	WIAF   WIAF-2208
9	67.40 cR from top of Chr9 link	1319	WIAF   WIAF-2209
9	68.80 cR from top of Chr9 link	1874	WIAF   WIAF-3813
9	68.9 cR from top of Chr9 link	932	WIAF   WIAF-1280
10	69.70 cR from top of Chr9 link	3433	WIAF   WIAF-1888
9	74.9 cR from top of Chr9 link	3444	WIAF   WIAF-1899
9	74.9 cR from top of Chr9 link	3445	WIAF   WIAF-1900
9	82.30 cR from top of Chr9 link	1246	WIAF   WIAF-2133
9	83.90 cR from top of Chr9 link	1278	WIAF   WIAF-2165
15	84.50 cR from top of Chr9 link	3482	WIAF   WIAF-1937
9	84.60 cR from top of Chr9 link	1579	WIAF   WIAF-3518
9	84.60 cR from top of Chr9 link	1581	WIAF   WIAF-3520
9	86.80 cR from top of Chr9 link	1192	WIAF   WIAF-2079
9	95.90 cR from top of Chr9 link	1892	WIAF   WIAF-3831
20	100.60 cR from top of Chr9 lin	3311	WIAF   WIAF-1764
9	100.60 cR from top of Chr9 lin	1405	WIAF   WIAF-3325
9	105.20 cR from top of Chr9 lin	1615	WIAF   WIAF-3554
9	105.80 cR from top of Chr9 lin	1237	WIAF   WIAF-2124
9	105.80 cR from top of Chr9 lin	1238	WIAF   WIAF-2125
25	106.20 cR from top of Chr9 lin	686	WIAF   WIAF-1177
9	106.20 cR from top of Chr9 lin	687	WIAF   WIAF-1178
9	109.3 cR from top of Chr9 link	2237	WIAF   WIAF-674
9	109.3 cR from top of Chr9 link	2238	WIAF   WIAF-675
9	118.20 cR from top of Chr9 lin	2881	WIAF   WIAF-779
30	122.20 cR from top of Chr9 lin	1536	WIAF   WIAF-3475
9	122.20 cR from top of Chr9 lin	1538	WIAF   WIAF-3477
9	123.20 cR from top of Chr9 lin	1430	WIAF   WIAF-3358
9	124.00 cR from top of Chr9 lin	1268	WIAF   WIAF-2155
9	124.00 cR from top of Chr9 lin	1269	WIAF   WIAF-2156
35	129.0 cR from top of Chr9 link	3830	WIAF   WIAF-2599
9	132.60 cR from top of Chr9 lin	1999	WIAF   WIAF-3938
9	136.30 cR from top of Chr9 lin	1154	WIAF   WIAF-1638
9	137.00 cR from top of Chr9 lin	1533	WIAF   WIAF-3472
9	137.70 cR from top of Chr9 lin	1301	WIAF   WIAF-2190
40	137.70 cR from top of Chr9 lin	1302	WIAF   WIAF-2191
9	138.0 cR from top of Chr9 link	2222	WIAF   WIAF-649
9	142.10 cR from top of Chr9 lin	1640	WIAF   WIAF-3579
9	142.70 cR from top of Chr9 lin	1331	WIAF   WIAF-2221
9	142.70 cR from top of Chr9 lin	1929	WIAF   WIAF-3868
45	143.50 cR from top of Chr9 lin	1207	WIAF   WIAF-2094
9	143.50 cR from top of Chr9 lin	1208	WIAF   WIAF-2095
9	143.90 cR from top of Chr9 lin	2558	WIAF   WIAF-182
9	144.60 cR from top of Chr9 lin	1797	WIAF   WIAF-3736
9	148.7 cR from top of Chr9 link	891	WIAF   WIAF-1136
50	148.7 cR from top of Chr9 link	1042	WIAF   WIAF-4131

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
9	164.70 cR from top of Chr9 lin	1994	WIAF   WIAF-3933
9	166.50 cR from top of Chr9 lin	1829	WIAF   WIAF-3768
9	210.3 cR from top of Chr9 link	3074	WIAF   WIAF-975
9	264.4 cR from top of Chr9 link	786	WIAF   WIAF-2049
5	293.7 cR from top of Chr9 link	2245	WIAF   WIAF-689
9	326.9 cR from top of Chr9 link	2621	WIAF   WIAF-277
9	328.5 cR from top of Chr9 link	2642	WIAF   WIAF-309
9	328.5 cR from top of Chr9 link	2725	WIAF   WIAF-430
9	336.4 cR from top of Chr9 link	2439	WIAF   WIAF-24
10	342.6 cR from top of Chr9 link	2341	WIAF   WIAF-2567
9	345.4 cR from top of Chr9 link	3059	WIAF   WIAF-960
9	367.4 cR from top of Chr9 link	2899	WIAF   WIAF-797
9	374.1 cR from top of Chr9 link	2453	WIAF   WIAF-41
9	389.8 cR from top of Chr9 link	2845	WIAF   WIAF-691
15	409.6 cR from top of Chr9 link	2145	WIAF   WIAF-433
9	437.9 cR from top of Chr9 link	2806	WIAF   WIAF-578
9	447.0 cR from top of Chr9 link	3050	WIAF   WIAF-951
9	449.9 cR from top of Chr9 link	3005	WIAF   WIAF-905
9	450.0 cR from top of Chr9 link	2601	WIAF   WIAF-245
20	480.5 cR from top of Chr9 link	2446	WIAF   WIAF-34
9	483.4 cR from top of Chr9 link	2565	WIAF   WIAF-194
9	493.6 cR from top of Chr9 link	2547	WIAF   WIAF-168
9	511.3 cR from top of Chr9 link	3468	WIAF   WIAF-1923
9	512.4 cR from top of Chr9 link	2521	WIAF   WIAF-130
25	515.6 cR from top of Chr9 link	2524	WIAF   WIAF-133
9	516.3 cR from top of Chr9 link	2102	WIAF   WIAF-166
9	518.9 cR from top of Chr9 link	3816	WIAF   WIAF-2570
9	523.2 cR from top of Chr9 link	2489	WIAF   WIAF-87
9	526.5 cR from top of Chr9 link	998	WIAF   WIAF-2346
30	526.5 cR from top of Chr9 link	999	WIAF   WIAF-2349
9	526.5 cR from top of Chr9 link	1000	WIAF   WIAF-2353
9	526.5 cR from top of Chr9 link	1001	WIAF   WIAF-2355
9		4226	MARSHFIELD   MID-18
9		4227	MARSHFIELD   MID-19
35	9	3995	SHGC/AFFYMETRIX   SNP-SHGC-10262
9		4007	SHGC/AFFYMETRIX   SNP-SHGC-1334
9		4071	SHGC/AFFYMETRIX   SNP-SHGC-14625
9		4075	SHGC/AFFYMETRIX   SNP-SHGC-15679
9		4079	SHGC/AFFYMETRIX   SNP-SHGC-16528
40	9	4014	SHGC/AFFYMETRIX   SNP-SHGC-3934
9		3150	WIAF   WIAF-1051
9		3210	WIAF   WIAF-1563
9		3580	WIAF   WIAF-2035
9		3581	WIAF   WIAF-2036
45	9	1588	WIAF   WIAF-3527
9		1862	WIAF   WIAF-3801
9		2465	WIAF   WIAF-58
9		2466	WIAF   WIAF-59
9		2477	WIAF   WIAF-73
50	9	3087	WIAF   WIAF-988

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
10	-6 cM	4315	UWGC   134
10	6.10 cR from top of Chr10 link	1310	WIAF   WIAF-2199
10	17.30 cR from top of Chr10 lin	1598	WIAF   WIAF-3537
5	17.30 cR from top of Chr10 lin	1600	WIAF   WIAF-3539
10	19.70 cR from top of Chr10 lin	1271	WIAF   WIAF-2158
10	22.20 cR from top of Chr10 lin	2538	WIAF   WIAF-152
10	28.50 cR from top of Chr10 lin	3181	WIAF   WIAF-1497
10	28.50 cR from top of Chr10 lin	3182	WIAF   WIAF-1498
10	29.00 cR from top of Chr10 lin	4576	HU-CHINA   10-1729
10	29.00 cR from top of Chr10 lin	3276	WIAF   WIAF-1729
10	31.40 cR from top of Chr10 lin	2016	WIAF   WIAF-1511
10	31.80 cR from top of Chr10 lin	1603	WIAF   WIAF-3542
10	32.00 cR from top of Chr10 lin	3265	WIAF   WIAF-1704
15	36.30 cR from top of Chr10 lin	2680	WIAF   WIAF-363
10	41.10 cR from top of Chr10 lin	3268	WIAF   WIAF-1713
10	43.3 cR from top of Chr10 link	3289	WIAF   WIAF-1742
10	43.80 cR from top of Chr10 lin	1675	WIAF   WIAF-3614
10	44.90 cR from top of Chr10 lin	1820	WIAF   WIAF-3759
20	45.10 cR from top of Chr10 lin	1969	WIAF   WIAF-3908
10	45.50 cR from top of Chr10 lin	1183	WIAF   WIAF-1715
10	45.50 cR from top of Chr10 lin	1184	WIAF   WIAF-1716
10	52.00 cR from top of Chr10 lin	1363	WIAF   WIAF-3266
10	61.60 cR from top of Chr10 lin	1178	WIAF   WIAF-1707
25	67.90 cR from top of Chr10 lin	1858	WIAF   WIAF-3797
10	79.40 cR from top of Chr10 lin	1962	WIAF   WIAF-3901
10	80.20 cR from top of Chr10 lin	3230	WIAF   WIAF-1632
10	83.30 cR from top of Chr10 lin	1960	WIAF   WIAF-3899
10	85.60 cR from top of Chr10 lin	1772	WIAF   WIAF-3711
30	89.40 cR from top of Chr10 lin	3517	WIAF   WIAF-1972
10	96.30 cR from top of Chr10 lin	1198	WIAF   WIAF-2085
10	96.90 cR from top of Chr10 lin	3213	WIAF   WIAF-1585
10	96.90 cR from top of Chr10 lin	1542	WIAF   WIAF-3481
10	96.90 cR from top of Chr10 lin	1651	WIAF   WIAF-3590
35	97.40 cR from top of Chr10 lin	1698	WIAF   WIAF-3637
10	97.60 cR from top of Chr10 lin	1554	WIAF   WIAF-3493
10	97.60 cR from top of Chr10 lin	1556	WIAF   WIAF-3495
10	105.3 cR from top of Chr10 lin	2428	WIAF   WIAF-9
10	106.70 cR from top of Chr10 li	3187	WIAF   WIAF-1503
40	107.90 cR from top of Chr10 li	1597	WIAF   WIAF-3536
10	110.30 cR from top of Chr10 li	1499	WIAF   WIAF-3438
10	110.50 cR from top of Chr10 li	3561	WIAF   WIAF-2016
10	112.50 cR from top of Chr10 li	1390	WIAF   WIAF-3295
10	112.70 cR from top of Chr10 li	1413	WIAF   WIAF-3334
45	112.70 cR from top of Chr10 li	1414	WIAF   WIAF-3335
10	113.50 cR from top of Chr10 li	1752	WIAF   WIAF-3691
10	122.50 cR from top of Chr10 li	1462	WIAF   WIAF-3396
10	123 cM	4321	UWGC   140
50	123.00 cR from top of Chr10 li	1703	WIAF   WIAF-3642
50	130.50 cR from top of Chr10 li	1762	WIAF   WIAF-3701

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	10 130.50 cR from top of Chr10 li 1764	WIAF	WIAF-3703
	10 133.6 cR from top of Chr10 lin 3035	WIAF	WIAF-935
	10 134.80 cR from top of Chr10 li 2045	WIAF	WIAF-1661
	10 134.80 cR from top of Chr10 li 1389	WIAF	WIAF-3294
5	10 138.90 cR from top of Chr10 li 1165	WIAF	WIAF-1676
	10 146.60 cR from top of Chr10 li 1394	WIAF	WIAF-3299
	10 146.80 cR from top of Chr10 li 1822	WIAF	WIAF-3761
	10 150.50 cR from top of Chr10 li 3412	WIAF	WIAF-1867
	10 155.30 cR from top of Chr10 li 3337	WIAF	WIAF-1790
10	10 180.3 cR from top of Chr10 lin 3065	WIAF	WIAF-966
	10 185.3 cR from top of Chr10 lin 2938	WIAF	WIAF-837
	10 293.4 cR from top of Chr10 lin 798	WIAF	WIAF-4055
	10 306.5 cR from top of Chr10 lin 2552	WIAF	WIAF-175
	10 343.7 cR from top of Chr10 lin 939	WIAF	WIAF-1297
15	10 356.8 cR from top of Chr10 lin 2199	WIAF	WIAF-609
	10 359.1 cR from top of Chr10 lin 3529	WIAF	WIAF-1984
	10 366.6 cR from top of Chr10 lin 3141	WIAF	WIAF-1042
	10 382.1 cR from top of Chr10 lin 2127	WIAF	WIAF-303
	10 384.4 cR from top of Chr10 lin 2247	WIAF	WIAF-692
20	10 389.5 cR from top of Chr10 lin 2536	WIAF	WIAF-149
	10 425.7 cR from top of Chr10 lin 2912	WIAF	WIAF-811
	10 431.3 cR from top of Chr10 lin 2641	WIAF	WIAF-308
	10 433.0 cR from top of Chr10 lin 2081	WIAF	WIAF-31
	10 437.2 cR from top of Chr10 lin 2128	WIAF	WIAF-310
25	10 440.2 cR from top of Chr10 lin 3346	WIAF	WIAF-1799
	10 442.3 cR from top of Chr10 lin 2277	WIAF	WIAF-744
	10 467.6 cR from top of Chr10 lin 3434	WIAF	WIAF-1889
	10 467.6 cR from top of Chr10 lin 3435	WIAF	WIAF-1890
	10 496.3 cR from top of Chr10 lin 2991	WIAF	WIAF-891
30	10 505.8 cR from top of Chr10 lin 2351	WIAF	WIAF-2588
	10 505.8 cR from top of Chr10 lin 2353	WIAF	WIAF-2593
	10 515.2 cR from top of Chr10 lin 2882	WIAF	WIAF-780
	10 515.7 cR from top of Chr10 lin 3334	WIAF	WIAF-1787
	10 537.8 cR from top of Chr10 lin 3416	WIAF	WIAF-1871
35	10 542.2 cR from top of Chr10 lin 3037	WIAF	WIAF-937
	10 551.7 cR from top of Chr10 lin 3102	WIAF	WIAF-1003
	10 557.3 cR from top of Chr10 lin 3506	WIAF	WIAF-1961
	10 558.3 cR from top of Chr10 lin 3155	WIAF	WIAF-1056
	10 567.5 cR from top of Chr10 lin 3853	WIAF	WIAF-2640
40	10 598.4 cR from top of Chr10 lin 2620	WIAF	WIAF-271
	10 620.5 cR from top of Chr10 lin 3558	WIAF	WIAF-2013
	10 623.8 cR from top of Chr10 lin 2879	WIAF	WIAF-777
	10 646.1 cR from top of Chr10 lin 894	WIAF	WIAF-1142
	10 4153 SHGC/AFFYMETRIX		SNP-SHGC-14257
45	10 4159 SHGC/AFFYMETRIX		SNP-SHGC-14726
	10 4076 SHGC/AFFYMETRIX		SNP-SHGC-15732
	10 4166 SHGC/AFFYMETRIX		SNP-SHGC-23692
	10 4103 SHGC/AFFYMETRIX		SNP-SHGC-30908
	10 4104 SHGC/AFFYMETRIX		SNP-SHGC-31374
50	10 3976 SHGC/AFFYMETRIX		SNP-SHGC-35401

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	dbSNP	HANDLE   LOCAL
		<u>ASSAY ID</u>	<u>SNP ID</u>
	10	3724	WIAF   WIAF-2452
	10	1530	WIAF   WIAF-3469
	10	1691	WIAF   WIAF-3630
	10	3882	WIAF   WIAF-3943
5	10	2483	WIAF   WIAF-80
	10	3001	WIAF   WIAF-901
	10	3079	WIAF   WIAF-980
	11 3.10 cR from top of Chr11 link	1544	WIAF   WIAF-3483
10	11 3.10 cR from top of Chr11 link	1683	WIAF   WIAF-3622
	11 3.90 cR from top of Chr11 link	685	WIAF   WIAF-1160
	11 3.90 cR from top of Chr11 link	694	WIAF   WIAF-1245
	11 4.60 cR from top of Chr11 link	1123	WIAF   WIAF-1566
	11 15.50 cR from top of Chr11 lin	1900	WIAF   WIAF-3839
15	11 15.60 cR from top of Chr11 lin	665	WIAF   WIAF-1061
	11 15.60 cR from top of Chr11 lin	1710	WIAF   WIAF-3649
	11 16.80 cR from top of Chr11 lin	1392	WIAF   WIAF-3297
	11 18.50 cR from top of Chr11 lin	1568	WIAF   WIAF-3507
	11 18.50 cR from top of Chr11 lin	1571	WIAF   WIAF-3510
20	11 23.50 cR from top of Chr11 lin	699	WIAF   WIAF-1271
	11 23.60 cR from top of Chr11 lin	2987	WIAF   WIAF-887
	11 23.70 cR from top of Chr11 lin	1580	WIAF   WIAF-3519
	11 24.50 cR from top of Chr11 lin	1355	WIAF   WIAF-3258
	11 26.50 cR from top of Chr11 lin	1553	WIAF   WIAF-3492
25	11 30.7 cR from top of Chr11 link	2615	WIAF   WIAF-262
	11 34.9 cR from top of Chr11 link	784	WIAF   WIAF-2043
	11 38.00 cR from top of Chr11 lin	3494	WIAF   WIAF-1949
	11 38.3 cR from top of Chr11 link	2921	WIAF   WIAF-820
	11 39.80 cR from top of Chr11 lin	1531	WIAF   WIAF-3470
30	11 43.7 cR from top of Chr11 link	3316	WIAF   WIAF-1769
	11 45.7 cR from top of Chr11 link	3400	WIAF   WIAF-1855
	11 52.1 cR from top of Chr11 link	2192	WIAF   WIAF-591
	11 52.2 cR from top of Chr11 link	2176	WIAF   WIAF-551
	11 58.7 cR from top of Chr11 link	3502	WIAF   WIAF-1957
35	11 62.20 cR from top of Chr11 lin	1958	WIAF   WIAF-3897
	11 62.30 cR from top of Chr11 lin	1371	WIAF   WIAF-3274
	11 62.50 cR from top of Chr11 lin	1560	WIAF   WIAF-3499
	11 62.50 cR from top of Chr11 lin	1917	WIAF   WIAF-3856
	11 63.20 cR from top of Chr11 lin	3198	WIAF   WIAF-1535
40	11 63.20 cR from top of Chr11 lin	3199	WIAF   WIAF-1536
	11 67.00 cR from top of Chr11 lin	1277	WIAF   WIAF-2164
	11 68.0 cR from top of Chr11 link	2233	WIAF   WIAF-668
	11 68.30 cR from top of Chr11 lin	2056	WIAF   WIAF-1719
	11 68.30 cR from top of Chr11 lin	1330	WIAF   WIAF-2220
45	11 74.30 cR from top of Chr11 lin	3158	WIAF   WIAF-1059
	11 76.50 cR from top of Chr11 lin	1361	WIAF   WIAF-3264
	11 76.50 cR from top of Chr11 lin	1720	WIAF   WIAF-3659
	11 76.60 cR from top of Chr11 lin	1306	WIAF   WIAF-2195
	11 77.10 cR from top of Chr11 lin	1901	WIAF   WIAF-3840
50	11 77.50 cR from top of Chr11 lin	2022	WIAF   WIAF-1529

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
11	80.10 cR from top of Chr11 lin 1645	WIAF	WIAF-3584
11	81.90 cR from top of Chr11 lin 1202	WIAF	WIAF-2089
11	82.90 cR from top of Chr11 lin 2001	WIAF	WIAF-3940
11	83.20 cR from top of Chr11 lin 3091	WIAF	WIAF-992
5	84.20 cR from top of Chr11 lin 3170	WIAF	WIAF-1480
11	86.90 cR from top of Chr11 lin 3162	WIAF	WIAF-1465
11	89.8 cR from top of Chr11 link 996	WIAF	WIAF-2045
11	89.8 cR from top of Chr11 link 3585	WIAF	WIAF-2046
11	93.30 cR from top of Chr11 lin 1217	WIAF	WIAF-2104
10	94.10 cR from top of Chr11 lin 3497	WIAF	WIAF-1952
11	97.90 cR from top of Chr11 lin 1122	WIAF	WIAF-1564
11	98.40 cR from top of Chr11 lin 1095	WIAF	WIAF-1483
11	98.40 cR from top of Chr11 lin 1096	WIAF	WIAF-1484
11	102.60 cR from top of Chr11 li 3495	WIAF	WIAF-1950
15	106.6 cR from top of Chr11 lin 864	WIAF	WIAF-1075
11	106.80 cR from top of Chr11 li 1868	WIAF	WIAF-3807
11	106.80 cR from top of Chr11 li 1869	WIAF	WIAF-3808
11	107.90 cR from top of Chr11 li 3202	WIAF	WIAF-1542
11	108.00 cR from top of Chr11 li 2049	WIAF	WIAF-1688
20	108.10 cR from top of Chr11 li 1478	WIAF	WIAF-3417
11	112.50 cR from top of Chr11 li 1103	WIAF	WIAF-1525
11	113 cM 4311	UWGC	130
11	116.20 cR from top of Chr11 li 1909	WIAF	WIAF-3848
11	117.40 cR from top of Chr11 li 675	WIAF	WIAF-1440
25	118.40 cR from top of Chr11 li 1714	WIAF	WIAF-3653
11	118.40 cR from top of Chr11 li 1715	WIAF	WIAF-3654
11	120.00 cR from top of Chr11 li 1830	WIAF	WIAF-3769
11	120.00 cR from top of Chr11 li 1831	WIAF	WIAF-3770
11	120.10 cR from top of Chr11 li 1943	WIAF	WIAF-3882
30	120.70 cR from top of Chr11 li 3258	WIAF	WIAF-1694
11	126.00 cR from top of Chr11 li 1854	WIAF	WIAF-3793
11	126.60 cR from top of Chr11 li 1386	WIAF	WIAF-3291
11	131.70 cR from top of Chr11 li 1899	WIAF	WIAF-3838
11	145.60 cR from top of Chr11 li 1806	WIAF	WIAF-3745
35	149.80 cR from top of Chr11 li 3267	WIAF	WIAF-1712
11	150.6 cR from top of Chr11 lin 1089	WIAF	WIAF-3183
11	166.3 cR from top of Chr11 lin 2942	WIAF	WIAF-841
11	171.5 cR from top of Chr11 lin 3829	WIAF	WIAF-2598
11	305.1 cR from top of Chr11 lin 3831	WIAF	WIAF-2600
40	314.5 cR from top of Chr11 lin 2892	WIAF	WIAF-790
11	323.0 cR from top of Chr11 lin 2606	WIAF	WIAF-252
11	323.0 cR from top of Chr11 lin 2607	WIAF	WIAF-253
11	344.4 cR from top of Chr11 lin 3407	WIAF	WIAF-1862
11	359.1 cR from top of Chr11 lin 2530	WIAF	WIAF-141
45	359.1 cR from top of Chr11 lin 2531	WIAF	WIAF-142
11	359.1 cR from top of Chr11 lin 2532	WIAF	WIAF-143
11	376.1 cR from top of Chr11 lin 3411	WIAF	WIAF-1866
11	377.9 cR from top of Chr11 lin 2533	WIAF	WIAF-146
11	379.5 cR from top of Chr11 lin 2484	WIAF	WIAF-81
50	387.7 cR from top of Chr11 lin 2592	WIAF	WIAF-228

	<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	11	387.7 cR from top of Chr11 lin 2593	WIAF   2593	WIAF-229
	11	389.8 cR from top of Chr11 lin 2857	WIAF   2857	WIAF-750
	11	392.6 cR from top of Chr11 lin 2943	WIAF   2943	WIAF-842
	11	403.4 cR from top of Chr11 lin 3815	WIAF   3815	WIAF-2569
5	11	419.0 cR from top of Chr11 lin 2590	WIAF   2590	WIAF-226
	11	419.0 cR from top of Chr11 lin 2591	WIAF   2591	WIAF-227
	11	421.1 cR from top of Chr11 lin 3371	WIAF   3371	WIAF-1824
	11	428.6 cR from top of Chr11 lin 3069	WIAF   3069	WIAF-970
	11	432.6 cR from top of Chr11 lin 2103	WIAF   2103	WIAF-174
10	11	458.3 cR from top of Chr11 lin 3478	WIAF   3478	WIAF-1933
	11	466.7 cR from top of Chr11 lin 3608	WIAF   3608	WIAF-2322
	11	488.1 cR from top of Chr11 lin 3134	WIAF   3134	WIAF-1035
	11	506.0 cR from top of Chr11 lin 1086	WIAF   1086	WIAF-2071
	11	522.5 cR from top of Chr11 lin 2485	WIAF   2485	WIAF-82
15	11	573.0 cR from top of Chr11 lin 2736	WIAF   2736	WIAF-447
	11	604.0 cR from top of Chr11 lin 2727	WIAF   2727	WIAF-432
	11	604.0 cR from top of Chr11 lin 3070	WIAF   3070	WIAF-971
	11	624.2 cR from top of Chr11 lin 3379	WIAF   3379	WIAF-1832
	11	4228 MARSHFIELD   Mid-21		
20	11	3959 SHGC/AFFYMETRIX	SNP-SHGC-10796	
	11	3965 SHGC/AFFYMETRIX	SNP-SHGC-11902	
	11	4064 SHGC/AFFYMETRIX	SNP-SHGC-13369	
	11	4073 SHGC/AFFYMETRIX	SNP-SHGC-15155	
	11	4093 SHGC/AFFYMETRIX	SNP-SHGC-17309	
25	11	4013 SHGC/AFFYMETRIX	SNP-SHGC-3925	
	11	4046 SHGC/AFFYMETRIX	SNP-SHGC-9225	
	11	2429 WIAF   WIAF-10		
	11	1090 WIAF   WIAF-1463		
	11	1134 WIAF   WIAF-1581		
30	11	3214 WIAF   WIAF-1588		
	11	3259 WIAF   WIAF-1695		
	11	3313 WIAF   WIAF-1766		
	11	3314 WIAF   WIAF-1767		
	11	3543 WIAF   WIAF-1998		
35	11	1638 WIAF   WIAF-3577		
	11	1666 WIAF   WIAF-3605		
	11	3090 WIAF   WIAF-991		
	12	0.70 cR from top of Chr12 link 1662	WIAF   1662	WIAF-3601
40	12	10.70 cR from top of Chr12 lin 3553	WIAF   3553	WIAF-2008
	12	13.80 cR from top of Chr12 lin 3353	WIAF   3353	WIAF-1806
	12	15.10 cR from top of Chr12 lin 1573	WIAF   1573	WIAF-3512
	12	19.60 cR from top of Chr12 lin 3352	WIAF   3352	WIAF-1805
	12	20.40 cR from top of Chr12 lin 674	WIAF   674	WIAF-1430
45	12	28.30 cR from top of Chr12 lin 1967	WIAF   1967	WIAF-3906
	12	28.40 cR from top of Chr12 lin 2862	WIAF   2862	WIAF-759
	12	29.30 cR from top of Chr12 lin 1625	WIAF   1625	WIAF-3564
	12	31.80 cR from top of Chr12 lin 1848	WIAF   1848	WIAF-3787
	12	32 cm 4322 UWGC   141		
50	12	32.80 cR from top of Chr12 lin 2979	WIAF   2979	WIAF-879

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	12 40.50 cR from top of Chr12 lin 1391	WIAF	WIAF-3296
	12 52.40 cR from top of Chr12 lin 1976	WIAF	WIAF-3915
	12 53.70 cR from top of Chr12 lin 3477	WIAF	WIAF-1932
	12 53.90 cR from top of Chr12 lin 3569	WIAF	WIAF-2024
5	12 54.00 cR from top of Chr12 lin 1320	WIAF	WIAF-2210
	12 54.40 cR from top of Chr12 lin 3461	WIAF	WIAF-1916
	12 54.40 cR from top of Chr12 lin 3462	WIAF	WIAF-1917
	12 57.40 cR from top of Chr12 lin 3262	WIAF	WIAF-1698
	12 57.40 cR from top of Chr12 lin 3532	WIAF	WIAF-1987
10	12 57.40 cR from top of Chr12 lin 3533	WIAF	WIAF-1988
	12 62.80 cR from top of Chr12 lin 2051	WIAF	WIAF-1692
	12 62.90 cR from top of Chr12 lin 1692	WIAF	WIAF-3631
	12 65.70 cR from top of Chr12 lin 1800	WIAF	WIAF-3739
	12 66.90 cR from top of Chr12 lin 913	WIAF	WIAF-1192
15	12 67.00 cR from top of Chr12 lin 1632	WIAF	WIAF-3571
	12 67.20 cR from top of Chr12 lin 2046	WIAF	WIAF-1662
	12 69.60 cR from top of Chr12 lin 3597	WIAF	WIAF-2202
	12 69.90 cR from top of Chr12 lin 2561	WIAF	WIAF-188
	12 70.60 cR from top of Chr12 lin 3089	WIAF	WIAF-990
20	12 71.10 cR from top of Chr12 lin 3525	WIAF	WIAF-1980
	12 72.30 cR from top of Chr12 lin 2495	WIAF	WIAF-93
	12 74.50 cR from top of Chr12 lin 2050	WIAF	WIAF-1691
	12 75.40 cR from top of Chr12 lin 1853	WIAF	WIAF-3792
	12 75.80 cR from top of Chr12 lin 1313	WIAF	WIAF-2203
25	12 75.80 cR from top of Chr12 lin 1314	WIAF	WIAF-2204
	12 75.80 cR from top of Chr12 lin 1315	WIAF	WIAF-2205
	12 75.80 cR from top of Chr12 lin 1730	WIAF	WIAF-3669
	12 76.50 cR from top of Chr12 lin 1799	WIAF	WIAF-3738
	12 78.60 cR from top of Chr12 lin 3234	WIAF	WIAF-1643
30	12 78.60 cR from top of Chr12 lin 3235	WIAF	WIAF-1644
	12 79.10 cR from top of Chr12 lin 1705	WIAF	WIAF-3644
	12 80.10 cR from top of Chr12 lin 1409	WIAF	WIAF-3330
	12 83.4 cR from top of Chr12 link 3335	WIAF	WIAF-1788
	12 97.00 cR from top of Chr12 lin 3377	WIAF	WIAF-1830
35	12 100.9 cR from top of Chr12 lin 2507	WIAF	WIAF-113
	12 101.90 cR from top of Chr12 li 1610	WIAF	WIAF-3549
	12 103.9 cR from top of Chr12 lin 3034	WIAF	WIAF-934
	12 108.70 cR from top of Chr12 li 1397	WIAF	WIAF-3302
	12 111.0 cR from top of Chr12 lin 2689	WIAF	WIAF-374
40	12 116.60 cR from top of Chr12 li 1972	WIAF	WIAF-3911
	12 117.50 cR from top of Chr12 li 1991	WIAF	WIAF-3930
	12 119.00 cR from top of Chr12 li 676	WIAF	WIAF-1442
	12 119.00 cR from top of Chr12 li 1639	WIAF	WIAF-3578
	12 119.00 cR from top of Chr12 li 1642	WIAF	WIAF-3581
45	12 123.90 cR from top of Chr12 li 3554	WIAF	WIAF-2009
	12 134.40 cR from top of Chr12 li 3576	WIAF	WIAF-2031
	12 136.30 cR from top of Chr12 li 2452	WIAF	WIAF-40
	12 146.30 cR from top of Chr12 li 1685	WIAF	WIAF-3624
	12 146.30 cR from top of Chr12 li 1687	WIAF	WIAF-3626
50	12 152.2 cR from top of Chr12 lin 3317	WIAF	WIAF-1770

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	12 152.4 cR from top of Chr12 lin 959	WIAF	WIAF-1343
	12 152.4 cR from top of Chr12 lin 1008	WIAF	WIAF-4037
	12 165.90 cR from top of Chr12 li 2957	WIAF	WIAF-856
	12 169.10 cR from top of Chr12 li 1562	WIAF	WIAF-3501
5	12 169.10 cR from top of Chr12 li 1567	WIAF	WIAF-3506
	12 182.2 cR from top of Chr12 lin 2506	WIAF	WIAF-111
	12 239.3 cR from top of Chr12 lin 2385	WIAF	WIAF-2679
	12 295.9 cR from top of Chr12 lin 3044	WIAF	WIAF-945
	12 317.2 cR from top of Chr12 lin 2597	WIAF	WIAF-237
10	12 317.2 cR from top of Chr12 lin 2598	WIAF	WIAF-238
	12 323.2 cR from top of Chr12 lin 2549	WIAF	WIAF-170
	12 327.8 cR from top of Chr12 lin 2744	WIAF	WIAF-463
	12 364.1 cR from top of Chr12 lin 3055	WIAF	WIAF-956
	12 368.3 cR from top of Chr12 lin 2270	WIAF	WIAF-728
15	12 378.5 cR from top of Chr12 lin 2377	WIAF	WIAF-2658
	12 378.5 cR from top of Chr12 lin 2378	WIAF	WIAF-2661
	12 378.7 cR from top of Chr12 lin 2919	WIAF	WIAF-818
	12 390.2 cR from top of Chr12 lin 2927	WIAF	WIAF-826
	12 396.1 cR from top of Chr12 lin 3140	WIAF	WIAF-1041
20	12 396.2 cR from top of Chr12 lin 2883	WIAF	WIAF-781
	12 396.2 cR from top of Chr12 lin 2884	WIAF	WIAF-782
	12 419.5 cR from top of Chr12 lin 3151	WIAF	WIAF-1052
	12 419.5 cR from top of Chr12 lin 3152	WIAF	WIAF-1053
	12 439.4 cR from top of Chr12 lin 2583	WIAF	WIAF-216
25	12 476.9 cR from top of Chr12 lin 731	WIAF	WIAF-1088
	12 478.9 cR from top of Chr12 lin 2117	WIAF	WIAF-251
	12 483.3 cR from top of Chr12 lin 2137	WIAF	WIAF-377
	12 526.5 cR from top of Chr12 lin 3463	WIAF	WIAF-1918
	12 557.3 cR from top of Chr12 lin 2794	WIAF	WIAF-552
30	12 580.4 cR from top of Chr12 lin 3157	WIAF	WIAF-1058
	12 600.8 cR from top of Chr12 lin 2737	WIAF	WIAF-449
	12 603.7 cR from top of Chr12 lin 2681	WIAF	WIAF-364
	12 614.7 cR from top of Chr12 lin 745	WIAF	WIAF-1214
	12 617.8 cR from top of Chr12 lin 2690	WIAF	WIAF-375
35	12 621.0 cR from top of Chr12 lin 1261	WIAF	WIAF-2148
	12 627.9 cR from top of Chr12 lin 2814	WIAF	WIAF-607
	12 628.7 cR from top of Chr12 lin 2964	WIAF	WIAF-864
	12 644.5 cR from top of Chr12 lin 2180	WIAF	WIAF-562
	12 4118 SHGC/AFFYMETRIX		SNPA-SHGC-13972
40	12 4128 SHGC/AFFYMETRIX		SNPB-SHGC-13972
	12 4003 SHGC/AFFYMETRIX		SNP-SHGC-12981
	12 4066 SHGC/AFFYMETRIX		SNP-SHGC-13464
	12 4070 SHGC/AFFYMETRIX		SNP-SHGC-13943
	12 4163 SHGC/AFFYMETRIX		SNP-SHGC-14942
45	12 4078 SHGC/AFFYMETRIX		SNP-SHGC-16483
	12 4108 SHGC/AFFYMETRIX		SNP-SHGC-35771
	12 4036 SHGC/AFFYMETRIX		SNP-SHGC-7632
	12 4051 SHGC/AFFYMETRIX		SNP-SHGC-9454
	12 3498 WIAF   WIAF-1953		
50	12 3772 WIAF   WIAF-2500		

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	12	2988	WIAF   WIAF-888
	13 18.60 cR from top of Chr13 lin 1655	WIAF	WIAF-3594
	13 23.30 cR from top of Chr13 lin 1855	WIAF	WIAF-3794
5	13 24.00 cR from top of Chr13 lin 3527	WIAF	WIAF-1982
	13 24.00 cR from top of Chr13 lin 3528	WIAF	WIAF-1983
	13 27.70 cR from top of Chr13 lin 2044	WIAF	WIAF-1647
	13 41.20 cR from top of Chr13 lin 1251	WIAF	WIAF-2138
	13 42.30 cR from top of Chr13 lin 1438	WIAF	WIAF-3367
10	13 44.10 cR from top of Chr13 lin 3342	WIAF	WIAF-1795
	13 46.60 cR from top of Chr13 lin 3185	WIAF	WIAF-1501
	13 46.60 cR from top of Chr13 lin 3186	WIAF	WIAF-1502
	13 46.7 cR from top of Chr13 link 923	WIAF	WIAF-1227
	13 47.50 cR from top of Chr13 lin 2551	WIAF	WIAF-173
15	13 47.60 cR from top of Chr13 lin 3403	WIAF	WIAF-1858
	13 47.90 cR from top of Chr13 lin 1837	WIAF	WIAF-3776
	13 58.00 cR from top of Chr13 lin 1451	WIAF	WIAF-3384
	13 58.50 cR from top of Chr13 lin 1444	WIAF	WIAF-3374
	13 59.40 cR from top of Chr13 lin 1604	WIAF	WIAF-3543
20	13 59.80 cR from top of Chr13 lin 1422	WIAF	WIAF-3350
	13 59.80 cR from top of Chr13 lin 1424	WIAF	WIAF-3352
	13 59.80 cR from top of Chr13 lin 1425	WIAF	WIAF-3353
	13 62.00 cR from top of Chr13 lin 1897	WIAF	WIAF-3836
	13 66.20 cR from top of Chr13 lin 2542	WIAF	WIAF-159
25	13 69.80 cR from top of Chr13 lin 3196	WIAF	WIAF-1531
	13 72.00 cR from top of Chr13 lin 1317	WIAF	WIAF-2207
	13 72.50 cR from top of Chr13 lin 1453	WIAF	WIAF-3386
	13 76.1 cR from top of Chr13 link 3864	WIAF	WIAF-2657
	13 76.1 cR from top of Chr13 link 3865	WIAF	WIAF-2659
30	13 77.10 cR from top of Chr13 lin 1365	WIAF	WIAF-3268
	13 78.30 cR from top of Chr13 lin 1817	WIAF	WIAF-3756
	13 79.2 cR from top of Chr13 link 930	WIAF	WIAF-1270
	13 83.4 cR from top of Chr13 link 2610	WIAF	WIAF-256
	13 87.1 cR from top of Chr13 link 2047	WIAF	WIAF-1686
35	13 87.1 cR from top of Chr13 link 2048	WIAF	WIAF-1687
	13 89.10 cR from top of Chr13 lin 2930	WIAF	WIAF-829
	13 92.80 cR from top of Chr13 lin 1612	WIAF	WIAF-3551
	13 117.50 cR from top of Chr13 lin 1411	WIAF	WIAF-3332
	13 122.3 cR from top of Chr13 lin 3139	WIAF	WIAF-1040
40	13 125.1 cR from top of Chr13 lin 781	WIAF	WIAF-1455
	13 125.1 cR from top of Chr13 lin 782	WIAF	WIAF-1456
	13 125.1 cR from top of Chr13 lin 783	WIAF	WIAF-1457
	13 134.3 cR from top of Chr13 lin 3156	WIAF	WIAF-1057
	13 143.1 cR from top of Chr13 lin 2471	WIAF	WIAF-65
45	13 144.1 cR from top of Chr13 lin 2099	WIAF	WIAF-156
	13 144.1 cR from top of Chr13 lin 2100	WIAF	WIAF-157
	13 145.4 cR from top of Chr13 lin 3011	WIAF	WIAF-911
	13 145.4 cR from top of Chr13 lin 3012	WIAF	WIAF-912
	13 145.4 cR from top of Chr13 lin 3064	WIAF	WIAF-965
50	13 149.7 cR from top of Chr13 lin 3481	WIAF	WIAF-1936

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	13 152.4 cR from top of Chr13 lin 3137	WIAF	WIAF-1038
	13 192.1 cR from top of Chr13 lin 2786	WIAF	WIAF-540
	13 192.1 cR from top of Chr13 lin 2787	WIAF	WIAF-541
	13 195.4 cR from top of Chr13 lin 789	WIAF	WIAF-2062
5	13 275.9 cR from top of Chr13 lin 2197	WIAF	WIAF-600
	13 288.1 cR from top of Chr13 lin 2159	WIAF	WIAF-497
	13 295.6 cR from top of Chr13 lin 3842	WIAF	WIAF-2619
	13 296.6 cR from top of Chr13 lin 4562	HU-CHINA	13-401-1
		4229	MARSHFIELD   MID-22
10	13	4143	SHGC/AFFYMETRIX   SNP-SHGC-13649
	13	4146	SHGC/AFFYMETRIX   SNP-SHGC-13999
	13	4083	SHGC/AFFYMETRIX   SNP-SHGC-16887
	13	4096	SHGC/AFFYMETRIX   SNP-SHGC-18881
	13	4008	SHGC/AFFYMETRIX   SNP-SHGC-2426
15	13	4102	SHGC/AFFYMETRIX   SNP-SHGC-30142
	13	4022	SHGC/AFFYMETRIX   SNP-SHGC-4718
	13	4034	SHGC/AFFYMETRIX   SNP-SHGC-6784
	13	4039	SHGC/AFFYMETRIX   SNP-SHGC-8465
	13	3389	WIAF   WIAF-1843
20	13	2982	WIAF   WIAF-882
	13	2491	WIAF   WIAF-89
	13	2492	WIAF   WIAF-90
	13	3072	WIAF   WIAF-973
25			
	14 3.30 cR from top of Chr14 link 3304	WIAF	WIAF-1757
	14 3.30 cR from top of Chr14 link 3305	WIAF	WIAF-1758
	14 3.30 cR from top of Chr14 link 3306	WIAF	WIAF-1759
	14 3.30 cR from top of Chr14 link 3307	WIAF	WIAF-1760
30	14 5.90 cR from top of Chr14 link 2785	WIAF	WIAF-536
	14 13.5 cR from top of Chr14 link 2796	WIAF	WIAF-554
	14 17.00 cR from top of Chr14 lin 1948	WIAF	WIAF-3887
	14 20.6 cR from top of Chr14 link 4567	HU-CHINA	14-729
	14 20.6 cR from top of Chr14 link 2271	WIAF	WIAF-729
35	14 22.4 cR from top of Chr14 link 3845	WIAF	WIAF-2624
	14 27.7 cR from top of Chr14 link 2696	WIAF	WIAF-382
	14 27.7 cR from top of Chr14 link 2906	WIAF	WIAF-804
	14 32.10 cR from top of Chr14 lin 2915	WIAF	WIAF-814
	14 36.50 cR from top of Chr14 lin 1428	WIAF	WIAF-3356
40	14 36.50 cR from top of Chr14 lin 1429	WIAF	WIAF-3357
	14 37.00 cR from top of Chr14 lin 1709	WIAF	WIAF-3648
	14 37.10 cR from top of Chr14 lin 701	WIAF	WIAF-1296
	14 42.40 cR from top of Chr14 lin 1260	WIAF	WIAF-2147
	14 46.10 cR from top of Chr14 lin 1689	WIAF	WIAF-3628
45	14 53.80 cR from top of Chr14 lin 1834	WIAF	WIAF-3773
	14 54.60 cR from top of Chr14 lin 1379	WIAF	WIAF-3283
	14 55.20 cR from top of Chr14 lin 1701	WIAF	WIAF-3640
	14 59.70 cR from top of Chr14 lin 1961	WIAF	WIAF-3900
	14 62.00 cR from top of Chr14 lin 1838	WIAF	WIAF-3777
50	14 63.60 cR from top of Chr14 lin 3281	WIAF	WIAF-1734

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	14 66.50 cR from top of Chr14 lin 3206	WIAF	WIAF-1557
	14 66.50 cR from top of Chr14 lin 3207	WIAF	WIAF-1558
	14 66.80 cR from top of Chr14 lin 1402	WIAF	WIAF-3322
	14 66.80 cR from top of Chr14 lin 1406	WIAF	WIAF-3326
5	14 66.80 cR from top of Chr14 lin 1408	WIAF	WIAF-3329
	14 69.0 cR from top of Chr14 link 1080	WIAF	WIAF-2051
	14 71.50 cR from top of Chr14 lin 1561	WIAF	WIAF-3500
	14 75 cM	4309	UWGC   128
	14 86.30 cR from top of Chr14 lin 3390	WIAF	WIAF-1845
10	14 95.5 cR from top of Chr14 link 2900	WIAF	WIAF-798
	14 99.60 cR from top of Chr14 lin 1669	WIAF	WIAF-3608
	14 101.00 cR from top of Chr14 li 1137	WIAF	WIAF-1600
	14 101.00 cR from top of Chr14 li 1138	WIAF	WIAF-1601
	14 101.00 cR from top of Chr14 li 1139	WIAF	WIAF-1602
15	14 109.00 cR from top of Chr14 li 1539	WIAF	WIAF-3478
	14 109.00 cR from top of Chr14 li 1541	WIAF	WIAF-3480
	14 121.60 cR from top of Chr14 li 1398	WIAF	WIAF-3360
	14 124.20 cR from top of Chr14 li 1432	WIAF	WIAF-3360
	14 124.20 cR from top of Chr14 li 1631	WIAF	WIAF-3570
20	14 124.20 cR from top of Chr14 li 1803	WIAF	WIAF-3742
	14 124.20 cR from top of Chr14 li 1805	WIAF	WIAF-3744
	14 124.20 cR from top of Chr14 li 1908	WIAF	WIAF-3847
	14 125.8 cR from top of Chr14 lin 3545	WIAF	WIAF-2000
	14 126.2 cR from top of Chr14 lin 744	WIAF	WIAF-1211
25	14 141.7 cR from top of Chr14 lin 2249	WIAF	WIAF-694
	14 167.2 cR from top of Chr14 lin 751	WIAF	WIAF-1263
	14 168.5 cR from top of Chr14 lin 863	WIAF	WIAF-1073
	14 168.5 cR from top of Chr14 lin 1011	WIAF	WIAF-4046
	14 174.5 cR from top of Chr14 lin 3301	WIAF	WIAF-1754
30	14 179.1 cR from top of Chr14 lin 3542	WIAF	WIAF-1997
	14 197.4 cR from top of Chr14 lin 2640	WIAF	WIAF-307
	14 197.6 cR from top of Chr14 lin 3465	WIAF	WIAF-1920
	14 228.7 cR from top of Chr14 lin 2859	WIAF	WIAF-754
	14 248.8 cR from top of Chr14 lin 2709	WIAF	WIAF-402
35	14 252.9 cR from top of Chr14 lin 3135	WIAF	WIAF-1036
	14 252.9 cR from top of Chr14 lin 3136	WIAF	WIAF-1037
	14 253.1 cR from top of Chr14 lin 2924	WIAF	WIAF-823
	14 253.4 cR from top of Chr14 lin 3876	WIAF	WIAF-2677
	14 255.0 cR from top of Chr14 lin 3361	WIAF	WIAF-1814
40	14 255.1 cR from top of Chr14 lin 3325	WIAF	WIAF-1778
	14 255.3 cR from top of Chr14 lin 2602	WIAF	WIAF-246
	14 263.3 cR from top of Chr14 lin 2599	WIAF	WIAF-239
	14 278.2 cR from top of Chr14 lin 3132	WIAF	WIAF-1033
	14 298.2 cR from top of Chr14 lin 2240	WIAF	WIAF-680
45	14 308.8 cR from top of Chr14 lin 961	WIAF	WIAF-1352
	14 324.3 cR from top of Chr14 lin 2936	WIAF	WIAF-835
	14 324.3 cR from top of Chr14 lin 2937	WIAF	WIAF-836
	14 335.6 cR from top of Chr14 lin 4582	HU-CHINA	14-2041
	14 335.6 cR from top of Chr14 lin 4555	HU-CHINA	14-2041-2
50	14 335.6 cR from top of Chr14 lin 3583	WIAF	WIAF-2041

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
14	352.4 cR from top of Chr14 lin	3362	WIAF   WIAF-1815
14	355.5 cR from top of Chr14 lin	2653	WIAF   WIAF-327
14	355.6 cR from top of Chr14 lin	3440	WIAF   WIAF-1895
14	359.0 cR from top of Chr14 lin	3294	WIAF   WIAF-1747
5	14 363.7 cR from top of Chr14 lin	2647	WIAF   WIAF-318
	14	3997	SHGC/AFFYMETRIX   SNP-SHGC-1127
	14	4137	SHGC/AFFYMETRIX   SNP-SHGC-13065
	14	4157	SHGC/AFFYMETRIX   SNP-SHGC-14530
	14	4088	SHGC/AFFYMETRIX   SNP-SHGC-17097
10	14	4101	SHGC/AFFYMETRIX   SNP-SHGC-19244
	14	4168	SHGC/AFFYMETRIX   SNP-SHGC-23875
	14	4032	SHGC/AFFYMETRIX   SNP-SHGC-6098
	14	4045	SHGC/AFFYMETRIX   SNP-SHGC-9043
	14	3125	WIAF   WIAF-1026
15	14	666	WIAF   WIAF-1072
	14	3595	WIAF   WIAF-2187
	14	3596	WIAF   WIAF-2188
	14	1328	WIAF   WIAF-2218
	14	1589	WIAF   WIAF-3528
20	14	2728	WIAF   WIAF-434
	14	2729	WIAF   WIAF-435
	14	2974	WIAF   WIAF-874
	15 0.00 cR from top of Chr15 link	3577	WIAF   WIAF-2032
25	15 4.70 cR from top of Chr15 link	1924	WIAF   WIAF-3863
	15 5.40 cR from top of Chr15 link	1712	WIAF   WIAF-3651
	15 9.60 cR from top of Chr15 link	2880	WIAF   WIAF-778
	15 11.00 cR from top of Chr15 lin	696	WIAF   WIAF-1254
	15 13.7 cR from top of Chr15 link	2931	WIAF   WIAF-830
30	15 13.7 cR from top of Chr15 link	2932	WIAF   WIAF-831
	15 17.70 cR from top of Chr15 lin	710	WIAF   WIAF-1439
	15 21.70 cR from top of Chr15 lin	3547	WIAF   WIAF-2002
	15 22.90 cR from top of Chr15 lin	3153	WIAF   WIAF-1054
	15 25.50 cR from top of Chr15 lin	2904	WIAF   WIAF-802
35	15 28.9 cR from top of Chr15 link	2058	WIAF   WIAF-2543
	15 28.9 cR from top of Chr15 link	3032	WIAF   WIAF-932
	15 37.30 cR from top of Chr15 lin	1968	WIAF   WIAF-3907
	15 38.20 cR from top of Chr15 lin	2473	WIAF   WIAF-68
	15 42.0 cR from top of Chr15 link	2625	WIAF   WIAF-284
40	15 42.70 cR from top of Chr15 lin	2469	WIAF   WIAF-62
	15 46 cM	4304	UWGC   123
	15 46.20 cR from top of Chr15 lin	3231	WIAF   WIAF-1633
	15 46.30 cR from top of Chr15 lin	3513	WIAF   WIAF-1968
	15 46.30 cR from top of Chr15 lin	1670	WIAF   WIAF-3609
45	15 46.30 cR from top of Chr15 lin	2426	WIAF   WIAF-6
	15 46.40 cR from top of Chr15 lin	1944	WIAF   WIAF-3883
	15 46.8 cR from top of Chr15 link	2956	WIAF   WIAF-855
	15 46.90 cR from top of Chr15 lin	1382	WIAF   WIAF-3287
	15 47 cM	4305	UWGC   124
50	15 48.10 cR from top of Chr15 lin	1951	WIAF   WIAF-3890

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
15	48.20 cR from top of Chr15 lin	3232	WIAF   WIAF-1635
15	49.70 cR from top of Chr15 lin	695	WIAF   WIAF-1248
15	49.9 cR from top of Chr15 link	2893	WIAF   WIAF-791
15	53 cM	4318	UWGC   137
5	53.70 cR from top of Chr15 lin	3176	WIAF   WIAF-1491
	60.90 cR from top of Chr15 lin	1695	WIAF   WIAF-3634
	65.30 cR from top of Chr15 lin	1434	WIAF   WIAF-3362
	65.30 cR from top of Chr15 lin	1436	WIAF   WIAF-3364
	65.8 cR from top of Chr15 link	3093	WIAF   WIAF-994
10	65.8 cR from top of Chr15 link	3094	WIAF   WIAF-995
	70.7 cR from top of Chr15 link	3573	WIAF   WIAF-2028
	71.30 cR from top of Chr15 lin	3489	WIAF   WIAF-1944
	71.30 cR from top of Chr15 lin	3490	WIAF   WIAF-1945
	71.80 cR from top of Chr15 lin	3557	WIAF   WIAF-2012
15	72.20 cR from top of Chr15 lin	1578	WIAF   WIAF-3517
	72.70 cR from top of Chr15 lin	1680	WIAF   WIAF-3619
	73.70 cR from top of Chr15 lin	3175	WIAF   WIAF-1490
	74.90 cR from top of Chr15 lin	1974	WIAF   WIAF-3913
	75.50 cR from top of Chr15 lin	3452	WIAF   WIAF-1907
20	75.70 cR from top of Chr15 lin	3160	WIAF   WIAF-1459
	76.40 cR from top of Chr15 lin	1937	WIAF   WIAF-3876
	76.6 cR from top of Chr15 link	3719	WIAF   WIAF-2447
	77.30 cR from top of Chr15 lin	1835	WIAF   WIAF-3774
	77.40 cR from top of Chr15 lin	2015	WIAF   WIAF-1509
25	78.60 cR from top of Chr15 lin	1744	WIAF   WIAF-3683
	85.4 cR from top of Chr15 link	2101	WIAF   WIAF-163
	94.40 cR from top of Chr15 lin	1190	WIAF   WIAF-2077
	97.60 cR from top of Chr15 lin	1760	WIAF   WIAF-3699
	102.60 cR from top of Chr15 li	1648	WIAF   WIAF-3587
30	104.40 cR from top of Chr15 li	1910	WIAF   WIAF-3849
	105.4 cR from top of Chr15 lin	2646	WIAF   WIAF-317
	105.5 cR from top of Chr15 lin	2711	WIAF   WIAF-406
	108.70 cR from top of Chr15 li	1911	WIAF   WIAF-3850
	108.70 cR from top of Chr15 li	1914	WIAF   WIAF-3853
35	121.4 cR from top of Chr15 lin	3496	WIAF   WIAF-1951
	121.7 cR from top of Chr15 lin	3406	WIAF   WIAF-1861
	133.5 cR from top of Chr15 lin	3812	WIAF   WIAF-2564
	139.7 cR from top of Chr15 lin	2174	WIAF   WIAF-547
	142.1 cR from top of Chr15 lin	2097	WIAF   WIAF-140
40	144.2 cR from top of Chr15 lin	2660	WIAF   WIAF-336
	152.5 cR from top of Chr15 lin	2490	WIAF   WIAF-88
	159.6 cR from top of Chr15 lin	2345	WIAF   WIAF-2576
	180.8 cR from top of Chr15 lin	2679	WIAF   WIAF-362
	194.2 cR from top of Chr15 lin	2585	WIAF   WIAF-219
45	195.6 cR from top of Chr15 lin	3099	WIAF   WIAF-1000
	202.7 cR from top of Chr15 lin	2721	WIAF   WIAF-425
	207.0 cR from top of Chr15 lin	2539	WIAF   WIAF-153
	228.4 cR from top of Chr15 lin	3110	WIAF   WIAF-1011
	228.4 cR from top of Chr15 lin	3111	WIAF   WIAF-1012
50	228.6 cR from top of Chr15 lin	2949	WIAF   WIAF-848

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
15	247.4 cR from top of Chr15 lin	1053	WIAF   WIAF-4162
15	286.2 cR from top of Chr15 lin	2369	WIAF   WIAF-2630
15	306.5 cR from top of Chr15 lin	856	WIAF   WIAF-453
15	306.5 cR from top of Chr15 lin	2740	WIAF   WIAF-454
5	308.4 cR from top of Chr15 lin	3969	SHGC/AFFYMETRIX   SNP-SHGC-14665
	332.8 cR from top of Chr15 lin	2115	WIAF   WIAF-243
	332.8 cR from top of Chr15 lin	2116	WIAF   WIAF-244
	344.1 cR from top of Chr15 lin	2584	WIAF   WIAF-218
	355.1 cR from top of Chr15 lin	2733	WIAF   WIAF-443
10	355.1 cR from top of Chr15 lin	2734	WIAF   WIAF-444
	363.7 cR from top of Chr15 lin	3457	WIAF   WIAF-1912
	388.6 cR from top of Chr15 lin	2557	WIAF   WIAF-181
	396.8 cR from top of Chr15 lin	1054	WIAF   WIAF-4163
	396.8 cR from top of Chr15 lin	2980	WIAF   WIAF-880
15	15	4120	SNPA-SHGC-15063
		4130	SNPB-SHGC-15063
		4139	SNP-SHGC-13105
		4148	SNP-SHGC-14096
		4154	SNP-SHGC-14356
20	15	3971	SHGC/AFFYMETRIX   SNP-SHGC-17150
		4047	SHGC/AFFYMETRIX   SNP-SHGC-9310
		3386	WIAF   WIAF-1840
		3684	WIAF   WIAF-2412
	15	3761	WIAF   WIAF-2489
25	15	1774	WIAF   WIAF-3713
	15	1928	WIAF   WIAF-3867
	15	3906	WIAF   WIAF-3980
	15	3920	WIAF   WIAF-4007
	15	3025	WIAF   WIAF-925
30	15	3026	WIAF   WIAF-926
16	5.10 cR from top of Chr16 link	1151	WIAF   WIAF-1628
35	5.60 cR from top of Chr16 link	3191	WIAF   WIAF-1514
	5.80 cR from top of Chr16 link	1773	WIAF   WIAF-3712
	9.00 cR from top of Chr16 link	693	WIAF   WIAF-1244
	10.40 cR from top of Chr16 link	3024	WIAF   WIAF-924
	14.60 cR from top of Chr16 link	1404	WIAF   WIAF-3324
	15.50 cR from top of Chr16 link	1889	WIAF   WIAF-3828
40	20.60 cR from top of Chr16 link	1223	WIAF   WIAF-2110
	21.4 cR from top of Chr16 link	2710	WIAF   WIAF-403
	22.1 cR from top of Chr16 link	2766	WIAF   WIAF-506
	25.2 cR from top of Chr16 link	2481	WIAF   WIAF-77
	25.5 cR from top of Chr16 link	2891	WIAF   WIAF-789
45	30.60 cR from top of Chr16 link	3550	WIAF   WIAF-2005
	37.6 cR from top of Chr16 link	2760	WIAF   WIAF-495
	41.60 cR from top of Chr16 link	2036	WIAF   WIAF-1613
	42.70 cR from top of Chr16 link	1485	WIAF   WIAF-3424
	42.70 cR from top of Chr16 link	1489	WIAF   WIAF-3428
50	43.30 cR from top of Chr16 link	1649	WIAF   WIAF-3588

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
16	44.0 cR from top of Chr16 link 3021	WIAF	WIAF-921
16	69.70 cR from top of Chr16 lin 4566	HU-CHINA	16-1697
16	69.70 cR from top of Chr16 lin 3261	WIAF	WIAF-1697
16	70.3 cR from top of Chr16 link 2770	WIAF	WIAF-510
5	72.60 cR from top of Chr16 lin 1249	WIAF	WIAF-2136
16	74.20 cR from top of Chr16 lin 690	WIAF	WIAF-1210
16	75.80 cR from top of Chr16 lin 1965	WIAF	WIAF-3904
16	86.50 cR from top of Chr16 lin 1775	WIAF	WIAF-3714
16	87.50 cR from top of Chr16 lin 1704	WIAF	WIAF-3643
10	91.50 cR from top of Chr16 lin 1495	WIAF	WIAF-3434
16	91.50 cR from top of Chr16 lin 1496	WIAF	WIAF-3435
16	92.40 cR from top of Chr16 lin 3359	WIAF	WIAF-1812
16	97.90 cR from top of Chr16 lin 3166	WIAF	WIAF-1474
16	98.0 cR from top of Chr16 link 2759	WIAF	WIAF-494
15	98.10 cR from top of Chr16 lin 3454	WIAF	WIAF-1909
16	98.20 cR from top of Chr16 lin 1671	WIAF	WIAF-3610
16	103.10 cR from top of Chr16 li 1555	WIAF	WIAF-3494
16	103.10 cR from top of Chr16 li 1654	WIAF	WIAF-3593
16	107.60 cR from top of Chr16 li 3121	WIAF	WIAF-1022
20	109.20 cR from top of Chr16 li 1435	WIAF	WIAF-3363
16	109.20 cR from top of Chr16 li 1437	WIAF	WIAF-3366
16	109.20 cR from top of Chr16 li 1439	WIAF	WIAF-3368
16	109.40 cR from top of Chr16 li 2251	WIAF	WIAF-697
16	112.3 cR from top of Chr16 lin 2375	WIAF	WIAF-2652
25	113.8 cR from top of Chr16 lin 3057	WIAF	WIAF-958
16	113.9 cR from top of Chr16 lin 3046	WIAF	WIAF-947
16	113.9 cR from top of Chr16 lin 3047	WIAF	WIAF-948
16	119.10 cR from top of Chr16 li 1912	WIAF	WIAF-3851
16	119.10 cR from top of Chr16 li 1916	WIAF	WIAF-3855
30	122.1 cR from top of Chr16 lin 2791	WIAF	WIAF-546
16	123.30 cR from top of Chr16 li 2054	WIAF	WIAF-1717
16	123.30 cR from top of Chr16 li 2055	WIAF	WIAF-1718
16	130.80 cR from top of Chr16 li 1255	WIAF	WIAF-2142
16	130.80 cR from top of Chr16 li 1776	WIAF	WIAF-3715
35	131.4 cR from top of Chr16 lin 2989	WIAF	WIAF-889
16	140.1 cR from top of Chr16 lin 2122	WIAF	WIAF-285
16	227.3 cR from top of Chr16 lin 3409	WIAF	WIAF-1864
16	235.6 cR from top of Chr16 lin 3516	WIAF	WIAF-1971
16	242.9 cR from top of Chr16 lin 2241	WIAF	WIAF-681
40	242.9 cR from top of Chr16 lin 2242	WIAF	WIAF-682
16	305.1 cR from top of Chr16 lin 1270	WIAF	WIAF-2157
16	312.9 cR from top of Chr16 lin 3058	WIAF	WIAF-959
16	320.4 cR from top of Chr16 lin 2468	WIAF	WIAF-61
16	327.3 cR from top of Chr16 lin 3556	WIAF	WIAF-2011
45	330.5 cR from top of Chr16 lin 3860	WIAF	WIAF-2650
16	333.4 cR from top of Chr16 lin 2515	WIAF	WIAF-123
16	333.6 cR from top of Chr16 lin 2560	WIAF	WIAF-184
16	338.2 cR from top of Chr16 lin 2519	WIAF	WIAF-127
16	348.6 cR from top of Chr16 lin 892	WIAF	WIAF-1139
50	351.6 cR from top of Chr16 lin 2146	WIAF	WIAF-437

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
5	16 351.6 cR from top of Chr16 lin	2147	WIAF   WIAF-438
	16 351.6 cR from top of Chr16 lin	2182	WIAF   WIAF-564
	16	4230	MARSHFIELD   MID-23
	16	3966	SHGC/AFFYMETRIX   SNP-SHGC-12011
	16	4038	SHGC/AFFYMETRIX   SNP-SHGC-8152
	16	1146	WIAF   WIAF-1614
	16	3671	WIAF   WIAF-2399
	16	2066	WIAF   WIAF-2553
	16	3810	WIAF   WIAF-2562
	10 16	1482	WIAF   WIAF-3421
15	16	1486	WIAF   WIAF-3425
	16	1527	WIAF   WIAF-3466
	16	1565	WIAF   WIAF-3504
	16	2960	WIAF   WIAF-859
	16	2992	WIAF   WIAF-892
	17 0.60 cR from top of Chr17 link	2435	WIAF   WIAF-18
	17 0.60 cR from top of Chr17 link	3467	WIAF   WIAF-1922
	20 17 0.60 cR from top of Chr17 link	1399	WIAF   WIAF-3305
	17 1.40 cR from top of Chr17 link	1726	WIAF   WIAF-3665
	17 2.10 cR from top of Chr17 link	1108	WIAF   WIAF-1540
25	17 4.50 cR from top of Chr17 link	3115	WIAF   WIAF-1016
	17 5.90 cR from top of Chr17 link	3549	WIAF   WIAF-2004
	17 7.60 cR from top of Chr17 link	1741	WIAF   WIAF-3680
	17 7.60 cR from top of Chr17 link	2451	WIAF   WIAF-39
	17 15.00 cR from top of Chr17 lin	1240	WIAF   WIAF-2127
	17 16.30 cR from top of Chr17 lin	1586	WIAF   WIAF-3525
	17 16.50 cR from top of Chr17 lin	1175	WIAF   WIAF-1699
	30 17 16.80 cR from top of Chr17 lin	2035	WIAF   WIAF-1598
	17 16.80 cR from top of Chr17 lin	1721	WIAF   WIAF-3660
	17 16.80 cR from top of Chr17 lin	2460	WIAF   WIAF-51
35	17 19 cM	4313	UWGC   132
	17 29.30 cR from top of Chr17 lin	1812	WIAF   WIAF-3751
	17 33.5 cR from top of Chr17 link	2922	WIAF   WIAF-821
	17 36.40 cR from top of Chr17 lin	2553	WIAF   WIAF-176
	17 45.20 cR from top of Chr17 lin	1473	WIAF   WIAF-3410
	17 45.40 cR from top of Chr17 lin	1950	WIAF   WIAF-3889
	17 45.5 cR from top of Chr17 link	3018	WIAF   WIAF-918
	40 17 45.5 cR from top of Chr17 link	3041	WIAF   WIAF-942
	17 51.8 cR from top of Chr17 link	3062	WIAF   WIAF-963
	17 51.90 cR from top of Chr17 lin	709	WIAF   WIAF-1419
45	17 53.10 cR from top of Chr17 lin	2018	WIAF   WIAF-1519
	17 57.30 cR from top of Chr17 lin	3541	WIAF   WIAF-1996
	17 59.9 cR from top of Chr17 link	2846	WIAF   WIAF-699
	17 60.10 cR from top of Chr17 lin	1258	WIAF   WIAF-2145
	17 60.60 cR from top of Chr17 lin	1441	WIAF   WIAF-3370
	17 61.10 cR from top of Chr17 lin	1696	WIAF   WIAF-3635
	17 62.8 cR from top of Chr17 link	2564	WIAF   WIAF-193
	50 17 62.80 cR from top of Chr17 lin	3387	WIAF   WIAF-1841

	<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	17	63.10 cR from top of Chr17 lin 3183	WIAF	WIAF-1499
	17	63.40 cR from top of Chr17 lin 684	WIAF	WIAF-1108
	17	64.80 cR from top of Chr17 lin 1997	WIAF	WIAF-3936
	17	65.00 cR from top of Chr17 lin 1840	WIAF	WIAF-3779
5	17	65.00 cR from top of Chr17 lin 1841	WIAF	WIAF-3780
	17	66.10 cR from top of Chr17 lin 3453	WIAF	WIAF-1908
	17	67.00 cR from top of Chr17 lin 1637	WIAF	WIAF-3576
	17	68.10 cR from top of Chr17 lin 3242	WIAF	WIAF-1652
	17	73.00 cR from top of Chr17 lin 3350	WIAF	WIAF-1803
10	17	83.90 cR from top of Chr17 lin 3397	WIAF	WIAF-1852
	17	84.10 cR from top of Chr17 lin 1094	WIAF	WIAF-1479
	17	84.90 cR from top of Chr17 lin 700	WIAF	WIAF-1276
	17	84.90 cR from top of Chr17 lin 673	WIAF	WIAF-1376
	17	86.30 cR from top of Chr17 lin 671	WIAF	WIAF-1361
15	17	86.70 cR from top of Chr17 lin 1416	WIAF	WIAF-3343
	17	87.60 cR from top of Chr17 lin 1898	WIAF	WIAF-3837
	17	94.1 cR from top of Chr17 link 2278	WIAF	WIAF-746
	17	94.1 cR from top of Chr17 link 2279	WIAF	WIAF-747
	17	94.1 cR from top of Chr17 link 2280	WIAF	WIAF-748
20	17	103.5 cR from top of Chr17 lin 2087	WIAF	WIAF-101
	17	250.6 cR from top of Chr17 lin 3008	WIAF	WIAF-908
	17	304.7 cR from top of Chr17 lin 2975	WIAF	WIAF-875
	17	307.9 cR from top of Chr17 lin 3288	WIAF	WIAF-1741
	17	311.1 cR from top of Chr17 lin 2844	WIAF	WIAF-688
25	17	317.4 cR from top of Chr17 lin 2858	WIAF	WIAF-752
	17	329.4 cR from top of Chr17 lin 2861	WIAF	WIAF-758
	17	338.1 cR from top of Chr17 lin 2869	WIAF	WIAF-767
	17	338.6 cR from top of Chr17 lin 2567	WIAF	WIAF-196
	17	355.3 cR from top of Chr17 lin 2767	WIAF	WIAF-507
30	17	355.5 cR from top of Chr17 lin 3000	WIAF	WIAF-900
	17	371.5 cR from top of Chr17 lin 3821	WIAF	WIAF-2582
	17	445.5 cR from top of Chr17 lin 2842	WIAF	WIAF-684
	17	462.1 cR from top of Chr17 lin 3570	WIAF	WIAF-2025
	17		4053 SHGC/AFFYMETRIX	SNPA-SHGC-31580
35	17		4059 SHGC/AFFYMETRIX	SNPB-SHGC-31580
	17		4001 SHGC/AFFYMETRIX	SNP-SHGC-1216
	17		4006 SHGC/AFFYMETRIX	SNP-SHGC-1310
	17		4072 SHGC/AFFYMETRIX	SNP-SHGC-14793
	17		4092 SHGC/AFFYMETRIX	SNP-SHGC-17275
40	17		4165 SHGC/AFFYMETRIX	SNP-SHGC-18143
	17		4095 SHGC/AFFYMETRIX	SNP-SHGC-18839
	17		4015 SHGC/AFFYMETRIX	SNP-SHGC-3939
	17		3120 WIAF	WIAF-1021
	17		3127 WIAF	WIAF-1028
45	17		2550 WIAF	WIAF-171
	17		1335 WIAF	WIAF-2225
	17		3678 WIAF	WIAF-2406
	17		3777 WIAF	WIAF-2505
	17		3778 WIAF	WIAF-2506
50	17		3800 WIAF	WIAF-2529

CHROMOSOME	FINE MAP LOCATION	dbSNP ASSAY ID	HANDLE   LOCAL	
				SNP ID
	17	2463	WIAF	WIAF-55
	17	3073	WIAF	WIAF-974
5	18 7.40 cR from top of Chr18 link	2011	WIAF	WIAF-1505
	18 7.40 cR from top of Chr18 link	2012	WIAF	WIAF-1506
	18 7.90 cR from top of Chr18 link	1189	WIAF	WIAF-2076
	18 19.5 cR from top of Chr18 link	3834	WIAF	WIAF-2603
	18 20.90 cR from top of Chr18 lin	3226	WIAF	WIAF-1626
10	18 21.1 cR from top of Chr18 link	2820	WIAF	WIAF-621
	18 28.1 cR from top of Chr18 link	3848	WIAF	WIAF-2631
	18 32.1 cR from top of Chr18 link	2819	WIAF	WIAF-620
	18 35.0 cR from top of Chr18 link	4584	HU-CHINA	18-525
15	18 35.0 cR from top of Chr18 link	4557	HU-CHINA	18-525-2
	18 35.0 cR from top of Chr18 link	2163	WIAF	WIAF-525
	18 35.0 cR from top of Chr18 link	2164	WIAF	WIAF-526
	18 36.20 cR from top of Chr18 lin	3355	WIAF	WIAF-1808
	18 36.20 cR from top of Chr18 lin	3356	WIAF	WIAF-1809
20	18 43.10 cR from top of Chr18 lin	1250	WIAF	WIAF-2137
	18 45.4 cR from top of Chr18 link	2587	WIAF	WIAF-222
	18 45.6 cR from top of Chr18 link	3101	WIAF	WIAF-1002
	18 52.5 cR from top of Chr18 link	3027	WIAF	WIAF-927
	18 56.2 cR from top of Chr18 link	3278	WIAF	WIAF-1731
25	18 56.2 cR from top of Chr18 link	3279	WIAF	WIAF-1732
	18 56.2 cR from top of Chr18 link	3280	WIAF	WIAF-1733
	18 57.00 cR from top of Chr18 lin	1940	WIAF	WIAF-3879
	18 58.1 cR from top of Chr18 link	3100	WIAF	WIAF-1001
	18 61.50 cR from top of Chr18 lin	1127	WIAF	WIAF-1571
30	18 61.50 cR from top of Chr18 lin	1128	WIAF	WIAF-1572
	18 61.60 cR from top of Chr18 lin	3164	WIAF	WIAF-1468
	18 66.60 cR from top of Chr18 lin	2486	WIAF	WIAF-84
	18 66.70 cR from top of Chr18 lin	1501	WIAF	WIAF-3440
	18 66.70 cR from top of Chr18 lin	1719	WIAF	WIAF-3658
35	18 68.20 cR from top of Chr18 lin	2007	WIAF	WIAF-1471
	18 72.30 cR from top of Chr18 lin	3322	WIAF	WIAF-1775
	18 80.30 cR from top of Chr18 lin	1596	WIAF	WIAF-3535
	18 81.60 cR from top of Chr18 lin	1697	WIAF	WIAF-3636
	18 81.60 cR from top of Chr18 lin	1700	WIAF	WIAF-3639
40	18 109.00 cR from top of Chr18 li	2918	WIAF	WIAF-817
	18 202.8 cR from top of Chr18 lin	2436	WIAF	WIAF-21
	18 288.2 cR from top of Chr18 lin	989	WIAF	WIAF-1432
	18 288.2 cR from top of Chr18 lin	1016	WIAF	WIAF-4064
	18 321.0 cR from top of Chr18 lin	3358	WIAF	WIAF-1811
45	18 323.9 cR from top of Chr18 lin	2781	WIAF	WIAF-530
	18 337.2 cR from top of Chr18 lin	2093	WIAF	WIAF-112
	18 355.2 cR from top of Chr18 lin	910	WIAF	WIAF-1187
	18 355.2 cR from top of Chr18 lin	911	WIAF	WIAF-1188
	18 394.1 cR from top of Chr18 lin	2282	WIAF	WIAF-753
50	18 454.4 cR from top of Chr18 lin	2109	WIAF	WIAF-210
	18 455.8 cR from top of Chr18 lin	2252	WIAF	WIAF-700
	18 455.8 cR from top of Chr18 lin	3029	WIAF	WIAF-929

**MISSING AT THE TIME OF PUBLICATION**

**-153-**

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
19	88.00 cR from top of Chr19 lin	1628	WIAF   WIAF-3567
19	89.8 cR from top of Chr19 link	3039	WIAF   WIAF-940
19	95.40 cR from top of Chr19 lin	1723	WIAF   WIAF-3662
19	97.60 cR from top of Chr19 lin	1376	WIAF   WIAF-3280
5	97.90 cR from top of Chr19 lin	1668	WIAF   WIAF-3607
19	99.80 cR from top of Chr19 lin	1172	WIAF   WIAF-1689
19	100.30 cR from top of Chr19 li	1214	WIAF   WIAF-2101
19	103.60 cR from top of Chr19 li	1964	WIAF   WIAF-3903
19	104.90 cR from top of Chr19 li	2427	WIAF   WIAF-7
10	106.70 cR from top of Chr19 li	1584	WIAF   WIAF-3523
19	107.40 cR from top of Chr19 li	1487	WIAF   WIAF-3426
19	109.90 cR from top of Chr19 li	3534	WIAF   WIAF-1989
19	109.90 cR from top of Chr19 li	1476	WIAF   WIAF-3414
19	256.4 cR from top of Chr19 lin	2343	WIAF   WIAF-2574
15	280.9 cR from top of Chr19 lin	3493	WIAF   WIAF-1948
19	285.1 cR from top of Chr19 lin	2888	WIAF   WIAF-786
19	286.2 cR from top of Chr19 lin	2617	WIAF   WIAF-264
19	290.4 cR from top of Chr19 lin	2731	WIAF   WIAF-439
19	290.7 cR from top of Chr19 lin	2717	WIAF   WIAF-416
20	324.0 cR from top of Chr19 lin	3582	WIAF   WIAF-2037
19	324.0 cR from top of Chr19 lin	3077	WIAF   WIAF-978
19	325.6 cR from top of Chr19 lin	3854	WIAF   WIAF-2641
19	331.1 cR from top of Chr19 lin	2423	WIAF   WIAF-1
19	331.2 cR from top of Chr19 lin	2832	WIAF   WIAF-654
25	341.9 cR from top of Chr19 lin	3045	WIAF   WIAF-946
19	349.3 cR from top of Chr19 lin	4591	HU-CHINA   19-941
19	349.3 cR from top of Chr19 lin	4592	HU-CHINA   19-941-2
19	349.3 cR from top of Chr19 lin	3040	WIAF   WIAF-941
19	380.7 cR from top of Chr19 lin	3867	WIAF   WIAF-2662
30	382.3 cR from top of Chr19 lin	3372	WIAF   WIAF-1825
19	382.3 cR from top of Chr19 lin	3373	WIAF   WIAF-1826
19	382.8 cR from top of Chr19 lin	3851	WIAF   WIAF-2637
19	385.1 cR from top of Chr19 lin	3485	WIAF   WIAF-1940
19		4054	SHGC/AFFYMETRIX   SNPA-SHGC-35310
35	19	4060	SHGC/AFFYMETRIX   SNPB-SHGC-35310
19		3957	SHGC/AFFYMETRIX   SNPB-SHGC-9656
19		3963	SHGC/AFFYMETRIX   SNP-SHGC-11607
19		4068	SHGC/AFFYMETRIX   SNP-SHGC-13495
19		3174	WIAF   WIAF-1488
40	19	2038	WIAF   WIAF-1618
19		3239	WIAF   WIAF-1649
19		3253	WIAF   WIAF-1671
19		3787	WIAF   WIAF-2515
19		1740	WIAF   WIAF-3679
45	19	2952	WIAF   WIAF-851
19		2985	WIAF   WIAF-885
19		2986	WIAF   WIAF-886
19		2993	WIAF   WIAF-893
19		2994	WIAF   WIAF-894
50	19	3023	WIAF   WIAF-923

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	19	3071	WIAF   WIAF-972
	20 7.10 cR from top of Chr20 link	1242	WIAF   WIAF-2129
	20 8.20 cR from top of Chr20 link	2057	WIAF   WIAF-1720
5	20 9.30 cR from top of Chr20 link	1842	WIAF   WIAF-3781
	20 9.40 cR from top of Chr20 link	1232	WIAF   WIAF-2119
	20 9.80 cR from top of Chr20 link	1125	WIAF   WIAF-1568
	20 9.80 cR from top of Chr20 link	1126	WIAF   WIAF-1569
	20 9.80 cR from top of Chr20 link	3344	WIAF   WIAF-1797
10	20 10.1 cR from top of Chr20 link	2856	WIAF   WIAF-749
	20 10.10 cR from top of Chr20 lin	2494	WIAF   WIAF-92
	20 14.7 cR from top of Chr20 link	2432	WIAF   WIAF-15
	20 22.00 cR from top of Chr20 lin	1880	WIAF   WIAF-3819
	20 23.2 cR from top of Chr20 link	3551	WIAF   WIAF-2006
15	20 24.7 cR from top of Chr20 link	2745	WIAF   WIAF-464
	20 24.7 cR from top of Chr20 link	2746	WIAF   WIAF-465
	20 25.6 cR from top of Chr20 link	2851	WIAF   WIAF-730
	20 30.60 cR from top of Chr20 lin	3256	WIAF   WIAF-1684
	20 32.60 cR from top of Chr20 lin	1570	WIAF   WIAF-3509
20	20 32.60 cR from top of Chr20 lin	1572	WIAF   WIAF-3511
	20 35.10 cR from top of Chr20 lin	1650	WIAF   WIAF-3589
	20 36.80 cR from top of Chr20 lin	2040	WIAF   WIAF-1621
	20 39.90 cR from top of Chr20 lin	1241	WIAF   WIAF-2128
	20 41.20 cR from top of Chr20 lin	2002	WIAF   WIAF-3941
25	20 41.40 cR from top of Chr20 lin	2000	WIAF   WIAF-3939
	20 41.60 cR from top of Chr20 lin	1225	WIAF   WIAF-2112
	20 41.60 cR from top of Chr20 lin	2713	WIAF   WIAF-410
	20 41.70 cR from top of Chr20 lin	1786	WIAF   WIAF-3725
	20 42.20 cR from top of Chr20 lin	1988	WIAF   WIAF-3927
30	20 42.70 cR from top of Chr20 lin	3013	WIAF   WIAF-913
	20 47.80 cR from top of Chr20 lin	2887	WIAF   WIAF-785
	20 48.70 cR from top of Chr20 lin	1490	WIAF   WIAF-3429
	20 49 cM	4325	UWGC   144
	20 53.00 cR from top of Chr20 lin	2896	WIAF   WIAF-794
35	20 55.00 cR from top of Chr20 lin	1181	WIAF   WIAF-1711
	20 55.00 cR from top of Chr20 lin	2626	WIAF   WIAF-286
	20 55.40 cR from top of Chr20 lin	1758	WIAF   WIAF-3697
	20 62.40 cR from top of Chr20 lin	2009	WIAF   WIAF-1481
	20 63.30 cR from top of Chr20 lin	3564	WIAF   WIAF-2019
40	20 65.30 cR from top of Chr20 lin	3398	WIAF   WIAF-1853
	20 74.00 cR from top of Chr20 lin	1099	WIAF   WIAF-1515
	20 74.00 cR from top of Chr20 lin	1875	WIAF   WIAF-3814
	20 82.10 cR from top of Chr20 lin	3514	WIAF   WIAF-1969
	20 82.50 cR from top of Chr20 lin	1537	WIAF   WIAF-3476
45	20 82.80 cR from top of Chr20 lin	1978	WIAF   WIAF-3917
	20 86.00 cR from top of Chr20 lin	1745	WIAF   WIAF-3684
	20 88.30 cR from top of Chr20 lin	1507	WIAF   WIAF-3446
	20 89.5 cR from top of Chr20 link	2083	WIAF   WIAF-67
	20 91.2 cR from top of Chr20 link	2130	WIAF   WIAF-333
50	20 96.50 cR from top of Chr20 lin	1998	WIAF   WIAF-3937

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
20	96.50 cR from top of Chr20 lin 2971	WIAF	WIAF-871
20	106.6 cR from top of Chr20 lin 2501	WIAF	WIAF-100
20	310.1 cR from top of Chr20 lin 2283	WIAF	WIAF-756
20	316.2 cR from top of Chr20 lin 2441	WIAF	WIAF-26
5	318.0 cR from top of Chr20 lin 2628	WIAF	WIAF-288
	318.9 cR from top of Chr20 lin 3365	WIAF	WIAF-1818
	319.9 cR from top of Chr20 lin 3540	WIAF	WIAF-1995
	320.2 cR from top of Chr20 lin 2082	WIAF	WIAF-42
	320.6 cR from top of Chr20 lin 771	WIAF	WIAF-1402
10	334.7 cR from top of Chr20 lin 4577	HU-CHINA	20-1357
	334.7 cR from top of Chr20 lin 763	WIAF	WIAF-1357
	334.7 cR from top of Chr20 lin 822	WIAF	WIAF-4221
	343.6 cR from top of Chr20 lin 2871	WIAF	WIAF-769
	343.6 cR from top of Chr20 lin 2872	WIAF	WIAF-770
15	343.6 cR from top of Chr20 lin 2873	WIAF	WIAF-771
	343.6 cR from top of Chr20 lin 2874	WIAF	WIAF-772
	4086	SHGC/AFFYMETRIX	SNP-SHGC-16962
	4009	SHGC/AFFYMETRIX	SNP-SHGC-2774
	4010	SHGC/AFFYMETRIX	SNP-SHGC-2775
20	20	4033	SHGC/AFFYMETRIX   SNP-SHGC-6179
	3238	WIAF	WIAF-1648
	3699	WIAF	WIAF-2427
	3052	WIAF	WIAF-953
25	21 11.30 cR from top of Chr21 lin 1558	WIAF	WIAF-3497
	21 11.30 cR from top of Chr21 lin 1559	WIAF	WIAF-3498
	21 17.60 cR from top of Chr21 lin 1623	WIAF	WIAF-3562
	21 30.50 cR from top of Chr21 lin 1606	WIAF	WIAF-3545
	21 31.8 cR from top of Chr21 link 2243	WIAF	WIAF-683
30	21 32.50 cR from top of Chr21 lin 1856	WIAF	WIAF-3795
	21 35.10 cR from top of Chr21 lin 1557	WIAF	WIAF-3496
	21 35.30 cR from top of Chr21 lin 1569	WIAF	WIAF-3508
	21 38.20 cR from top of Chr21 lin 3488	WIAF	WIAF-1943
	21 38.30 cR from top of Chr21 lin 3197	WIAF	WIAF-1534
35	21 39.30 cR from top of Chr21 lin 1718	WIAF	WIAF-3657
	21 45.00 cR from top of Chr21 lin 1547	WIAF	WIAF-3486
	21 57.40 cR from top of Chr21 lin 3200	WIAF	WIAF-1537
	21 57.40 cR from top of Chr21 lin 3201	WIAF	WIAF-1538
	21 58.30 cR from top of Chr21 lin 1540	WIAF	WIAF-3479
40	21 58.30 cR from top of Chr21 lin 1543	WIAF	WIAF-3482
	21 59.30 cR from top of Chr21 lin 1324	WIAF	WIAF-2214
	21 59.30 cR from top of Chr21 lin 1325	WIAF	WIAF-2215
	21 59.60 cR from top of Chr21 lin 4570	HU-CHINA	21-899
	21 59.60 cR from top of Chr21 lin 689	WIAF	WIAF-1199
45	21 59.60 cR from top of Chr21 lin 1166	WIAF	WIAF-1678
	21 59.60 cR from top of Chr21 lin 1746	WIAF	WIAF-3685
	21 59.60 cR from top of Chr21 lin 1748	WIAF	WIAF-3687
	21 59.60 cR from top of Chr21 lin 1802	WIAF	WIAF-3741
	21 59.60 cR from top of Chr21 lin 2999	WIAF	WIAF-899
50	21 119.1 cR from top of Chr21 lin 2206	WIAF	WIAF-624

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	21 153.3 cR from top of Chr21 lin	3855	WIAF   WIAF-2643
	21 153.3 cR from top of Chr21 lin	3859	WIAF   WIAF-2648
	21 157.0 cR from top of Chr21 lin	2434	WIAF   WIAF-17
	21 233.7 cR from top of Chr21 lin	3438	WIAF   WIAF-1893
5	21	3989	SHGC/AFFYMETRIX   SNPA-SHGC-9556
	21	4141	SHGC/AFFYMETRIX   SNP-SHGC-13352
	21	4011	SHGC/AFFYMETRIX   SNP-SHGC-2811
	21	4211	SHGC/AFFYMETRIX   SNP-SHGC-51813
	21	4212	SHGC/AFFYMETRIX   SNP-SHGC-51844
10	21	4213	SHGC/AFFYMETRIX   SNP-SHGC-51849
	21	4214	SHGC/AFFYMETRIX   SNP-SHGC-51852
	21	3982	SHGC/AFFYMETRIX   SNP-SHGC-51888
	21	4215	SHGC/AFFYMETRIX   SNP-SHGC-51907
	21	4216	SHGC/AFFYMETRIX   SNP-SHGC-51925
15	21	4030	SHGC/AFFYMETRIX   SNP-SHGC-51941
	21	4217	SHGC/AFFYMETRIX   SNP-SHGC-51944
	21	4031	SHGC/AFFYMETRIX   SNP-SHGC-51951
	21	3184	WIAF   WIAF-1500
	21	1170	WIAF   WIAF-1682
20	21	1171	WIAF   WIAF-1683
	21	3402	WIAF   WIAF-1857
	21	3427	WIAF   WIAF-1882
	21	1212	WIAF   WIAF-2099
25	X 1.7 cR from top of ChrX linkag	2384	WIAF   WIAF-2675
	X 7.0 cR from top of ChrX linkag	2188	WIAF   WIAF-576
	X 7.4 cR from top of ChrX linkag	2096	WIAF   WIAF-137
	X 11.10 cR from top of ChrX link	1293	WIAF   WIAF-2180
	X 11.10 cR from top of ChrX link	1294	WIAF   WIAF-2181
30	X 15.0 cR from top of ChrX linka	3870	WIAF   WIAF-2669
	X 15.0 cR from top of ChrX linka	3075	WIAF   WIAF-976
	X 15.7 cR from top of ChrX linka	2148	WIAF   WIAF-440
	X 23.5 cR from top of ChrX linka	773	WIAF   WIAF-1404
	X 28.10 cR from top of ChrX link	2573	WIAF   WIAF-203
35	X 28.10 cR from top of ChrX link	1244	WIAF   WIAF-2131
	X 30.10 cR from top of ChrX link	1833	WIAF   WIAF-3772
	X 41.4 cR from top of ChrX linka	3303	WIAF   WIAF-1756
	X 43.4 cR from top of ChrX linka	2803	WIAF   WIAF-572
	X 43.4 cR from top of ChrX linka	2804	WIAF   WIAF-573
40	X 59.80 cR from top of ChrX link	3486	WIAF   WIAF-1941
	X 91.6 cR from top of ChrX linka	2608	WIAF   WIAF-254
	X 91.6 cR from top of ChrX linka	2609	WIAF   WIAF-255
	X 91.8 cR from top of ChrX linka	3408	WIAF   WIAF-1863
	X 92.20 cR from top of ChrX link	3417	WIAF   WIAF-1872
45	X 96.80 cR from top of ChrX link	3548	WIAF   WIAF-2003
	X 120.30 cR from top of ChrX lin	705	WIAF   WIAF-1356
	X 187.70 cR from top of ChrX lin	3290	WIAF   WIAF-1743
	X 187.70 cR from top of ChrX lin	3291	WIAF   WIAF-1744
	X 232.1 cR from top of ChrX link	2257	WIAF   WIAF-707
50	X 282.4 cR from top of ChrX link	761	WIAF   WIAF-1350

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>		<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
	X 285.4 cR from top of ChrX link	3030	WIAF	WIAF-930
	X 290.1 cR from top of ChrX link	2072	WIAF	WIAF-2
	X 294.0 cR from top of ChrX link	2778	WIAF	WIAF-522
	X 301.2 cR from top of ChrX link	2776	WIAF	WIAF-519
5	X 301.3 cR from top of ChrX link	871	WIAF	WIAF-1098
	X 301.3 cR from top of ChrX link	872	WIAF	WIAF-1099
	X 304.8 cR from top of ChrX link	3824	WIAF	WIAF-2589
	X 315.6 cR from top of ChrX link	3599	WIAF	WIAF-2269
	X 315.6 cR from top of ChrX link	3600	WIAF	WIAF-2270
10	X 331.3 cR from top of ChrX link	3104	WIAF	WIAF-1005
	X Xp21.2, 32.550 Mb	625	KWOK	Xp1226-1
	X Xp21.2, 32.550 Mb	624	KWOK	Xp1226-2
	X Xq22, 105.900 Mb	623	KWOK	Xq1136-1
	X Xq22, 106.300 Mb	661	KWOK	Xq544-1
15	X Xq22, 106.300 Mb	660	KWOK	Xq544-2
	X Xq24, 117.624 Mb	641	KWOK	Xq3562-1
	X Xq24, 117.685 Mb	646	KWOK	Xq3655-2
	X Xq24, 117.754 Mb	648	KWOK	Xq3656-1
	X Xq24, 117.754 Mb	649	KWOK	Xq3656-2
20	X Xq24, 117.754 Mb	647	KWOK	Xq3656-3
	X Xq25	590	KWOK	Xq3855-1
	X Xq25	591	KWOK	Xq3858-1
	X Xq25, 122.820 Mb	588	KWOK	Xq3847-1
	X Xq25, 124.300 Mb	593	KWOK	Xq3868-1
25	X Xq25, 124.362 Mb	654	KWOK	Xq3773-1
	X Xq25, 124.456 Mb	655	KWOK	Xq3774-1
	X Xq25, 124.456 Mb	656	KWOK	Xq3774-2
	X Xq25, 124.673 Mb	592	KWOK	Xq3862-1
	X Xq25, 124.860 Mb	645	KWOK	Xq3570-1
30	X Xq25, 124.860 Mb	644	KWOK	Xq3570-2
	X Xq25, 124.860 Mb	642	KWOK	Xq3570-3
	X Xq25, 124.860 Mb	643	KWOK	Xq3570-4
	X Xq25, 125.110	621	KWOK	Xq3813-1
	X Xq25, 125.110	622	KWOK	Xq3813-2
35	X Xq25, 126.091 Mb	587	KWOK	Xq3846-1
	X Xq25, 126.091 Mb	586	KWOK	Xq3846-2
	X Xq25, 126.257	617	KWOK	Xq3705-1
	X Xq25, 126.257	618	KWOK	Xq3705-2
	X Xq25, 126.296 Mb	657	KWOK	Xq3804-1
40	X Xq25, 126.387	619	KWOK	Xq3812-1
	X Xq25, 126.387	620	KWOK	Xq3812-2
	X Xq25, 126.687 Mb	589	KWOK	Xq3849-1
	X Xq25, 126.846 Mb	652	KWOK	Xq3699-1
	X Xq25, 126.846 Mb	653	KWOK	Xq3699-2
45	X Xq25, 126.954 Mb	658	KWOK	Xq3811-1
	X Xq25, 126.954 Mb	659	KWOK	Xq3811-2
	X Xq25, 127.053 Mb	650	KWOK	Xq3698-1
	X Xq25, 127.053 Mb	651	KWOK	Xq3698-2
	X Xq26	594	KWOK	Xq3871-1
50	X Xq26, 131.172 Mb	597	KWOK	Xq3879-1

	<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
5	X	Xq26, 131.990 Mb	634	KWOK   Xq3070-1
	X	Xq26, 131.990 Mb	635	KWOK   Xq3070-2
	X	Xq26, 136.541 Mb	662	KWOK   Xq3874-1
	X	Xq26, 136.631 Mb	595	KWOK   Xq3875-1
	X	Xq26, 136.631 Mb	596	KWOK   Xq3875-2
	X	Xq26, 137.977	608	KWOK   Xq3695-1
	X	Xq26, 137.977	609	KWOK   Xq3695-2
	X	Xq26, 137.977	611	KWOK   Xq3695-3
	X	Xq26, 137.977	610	KWOK   Xq3695-4
	10	Xq26, 138.062	612	KWOK   Xq3696-1
15	X	Xq26, 138.062	613	KWOK   Xq3696-2
	X	Xq26, 138.062	614	KWOK   Xq3696-3
	X	Xq26, 138.062	615	KWOK   Xq3696-4
	X	Xq26, 138.062	616	KWOK   Xq3696-5
	X	Xq27	598	KWOK   Xq3885-1
	X	Xq27	599	KWOK   Xq3885-2
	X	Xq27	600	KWOK   Xq3886-1
	X	Xq27	601	KWOK   Xq3886-2
	X	Xq27, 141.250 Mb	631	KWOK   Xq2904-1
	20	Xq27, 141.250 Mb	632	KWOK   Xq2904-2
25	X	Xq27, 141.250 Mb	633	KWOK   Xq2904-3
	X	Xq27, 141.499 Mb	663	KWOK   Xq3887-1
	X	Xq27, 141.499 Mb	664	KWOK   Xq3887-2
	X	Xq27, 141.580 Mb	602	KWOK   Xq3888-1
	X	Xq28	603	KWOK   Xq3555-1
	X	Xq28	604	KWOK   Xq3555-2
	X	Xq28	605	KWOK   Xq3555-3
	X	Xq28	606	KWOK   Xq3555-4
	X	Xq28	607	KWOK   Xq3555-5
	30	Xq28, 157.074 Mb	585	KWOK   Xq3841-1
35	X	Xq28, 157.123 Mb	583	KWOK   Xq3840-1
	X	Xq28, 157.123 Mb	584	KWOK   Xq3840-2
	X	Xq28, 157.939 Mb	640	KWOK   Xq3476-1
	X	Xq28, 157.939 Mb	639	KWOK   Xq3476-2
	X	Xq28, 158.055 Mb	637	KWOK   Xq3449-1
	X	Xq28, 158.059 Mb	638	KWOK   Xq3471-1
	X	Xq28, 158.237 Mb	630	KWOK   Xq2816-1
	X	Xq28, 158.265 Mb	636	KWOK   Xq3274-1
	X	Xq28, 158.490 Mb	626	KWOK   Xq1452-1
	40	Xq28, 158.490 Mb	627	KWOK   Xq1452-2
45	X	Xq28, 158.490 Mb	628	KWOK   Xq1452-3
	X	Xq28, 158.490 Mb	629	KWOK   Xq1452-4
	X		4099	SHGC/AFFYMETRIX   SNP-SHGC-18945
	X		2008	WIAF   WIAF-1472
	X		3271	WIAF   WIAF-1723
50	X		3272	WIAF   WIAF-1724
	X		3469	WIAF   WIAF-1924
	X		3602	WIAF   WIAF-2274
	X		3869	WIAF   WIAF-2666
			3036	WIAF   WIAF-936

<u>CHROMOSOME</u>	<u>FINE MAP LOCATION</u>	<u>dbSNP ASSAY ID</u>	<u>HANDLE   LOCAL SNP ID</u>
5	Y	3930	OEFNER   M2
	Y	3930	OEFNER   M2
	Y	3931	OEFNER   M3
	Y	3931	OEFNER   M3
	Y	3932	OEFNER   M4
	Y	3933	OEFNER   M5
	Y	3933	OEFNER   M5
	Y	3934	OEFNER   M6
	Y	3935	OEFNER   M7
	Y	3936	OEFNER   M8
10	Y	3937	OEFNER   M9
	Y	3938	OEFNER   M10
	Y	3939	OEFNER   M11
	Y	3940	OEFNER   M12
	Y	3941	OEFNER   M13
15	Y	3942	OEFNER   M14
	Y	3943	OEFNER   M15
	Y	3944	OEFNER   M16
	Y	3944	OEFNER   M16
	Y	3945	OEFNER   M17
20	Y	3946	OEFNER   M18
	Y	3947	OEFNER   M19
	Y	3948	OEFNER   M20
	Y	3949	OEFNER   M21
25	Y	3950	OEFNER   M22

**E. METHODS FOR REMOVING NUCLEIC ACID DUPLEX WITH ABNORMAL BASE-PAIRING**

30        Provided herein is a method for removing a nucleic acid duplex containing one or more abnormal base-pairing in a population of nucleic acid duplexes, which method comprises: a) contacting a population of nucleic acid duplexes having or suspected of having a nucleic acid duplex containing one or more abnormal base-pairing with a mutant DNA repair enzyme or complex thereof, wherein the mutant DNA repair enzyme or complex thereof has binding affinity for the abnormal base-pairing in the duplex but has attenuated catalytic activity and whereby the nucleic acid duplex containing one or more abnormal base-pairing binds to the mutant DNA repair enzyme or complex thereof to form a binding complex; and b)

35        removing the binding complex formed in step a) from the population of nucleic acid duplexes, thereby the nucleic acid duplex containing one or

40

more abnormal base-pairing is removed from the population of nucleic acid duplexes.

In a specific embodiment, a population of nucleic acid duplexes comprise DNA:DNA, DNA:RNA and RNA:RNA duplexes. Preferably, the  
5 population comprises DNA:DNA duplexes.

In another specific embodiment, the nucleic acid duplex to be removed from the population comprise a base-pair mismatch, a base insertion, a base deletion or a pyrimidine dimer. Preferably, the base-pair mismatch is a single base-pair mismatch.

10 In still another specific embodiment, the population of nucleic acid duplexes is produced by an enzymatic amplification. Preferably, the population of nucleic acid duplexes is produced by a polymerase chain reaction or a reaction utilizing reverse transcription and subsequent DNA amplification of one or more expressed RNA sequences.

15 The binding complex formed between the nucleic acid duplex containing one or more abnormal base-pairing and the mutant DNA repair enzyme or complex thereof can be removed from the population of nucleic acid duplexes by any methods known in the art. For example, the binding complex can be separated from the population by conventional  
20 separation methods such as electrophoresis, centrifugation, filtration and chromatograph. The separation can also be effected by affinity separation/purification, *i.e.*, using moieties that bind proteins but not nucleic acids. For example, antibodies that bind proteins generally but not nucleic acids can be used, antibodies that specifically bind the mutant  
25 DNA repair enzyme or complex thereof can be used. In addition, the mutant DNA repair enzyme or complex thereof can be labelled and/or tagged and the separation can be effected through the labels or tags.

**F. METHODS FOR DETECTING AND LOCALIZING ABNORMAL BASE-PAIRING IN NUCLEIC ACID DUPLEX**

30 Also provided herein is a method for detecting and localizing an abnormal base-pairing in a nucleic acid duplex by contacting a nucleic

acid duplex having or suspected of having an abnormal base-pairing with a mutant DNA repair enzyme or complex thereof, where the mutant DNA repair enzyme or complex thereof has binding affinity for the abnormal base-pairing in the duplex but has attenuated catalytic activity and

5 whereby the nucleic acid duplex containing an abnormal base-pairing binds to the mutant DNA repair enzyme or complex thereof to form a binding complex; subjecting the nucleic acid duplex to hydrolysis with an exonuclease under conditions such that the binding complex formed in the first step blocks hydrolysis; and then determining the location within

10 the nucleic acid duplex protected from the hydrolysis, thereby detecting and localizing the abnormal base-pairing in the nucleic acid duplex.

In a specific embodiment, the nucleic acid duplex to be assayed is a DNA:DNA, a DNA:RNA or a RNA:RNA duplex. Preferably, the nucleic acid duplex to be assayed is a DNA:DNA.

15 In another specific embodiment, the abnormal base-pairing to be detected and localized is a base-pair mismatch, a base insertion, a base deletion or a pyrimidine dimer. Preferably, the base-pair mismatch to be detected and localized is a single base-pair mismatch.

Any exonucleases can be used in the present methods. For example, the exonucleases with the following Genbank Accession Nos. can be used: AF194116 (Escherichia coli exonuclease X), AF191741 (Arabidopsis thaliana exonuclease RRP41 (RRP41)), AF013497 (Pyrococcus furiosus endo/exonuclease (fen-1)), AF058396 (Chlamydophila caviae strain GPIC ssDNA-specific exonuclease (recJ)), AF151105 (Homo sapiens 3'-5' exonuclease TREX1 mRNA), AF151108 (Mus musculus 3'-5' exonuclease TREX2), AF151107 (Homo sapiens 3'-5' exonuclease TREX2 mRNA), AF151106 (Mus musculus 3'-5' exonuclease TREX1), AF083915 (Chilo iridescent virus exonuclease II homolog (EX02)), AF140550 (Salmonella typhimurium exonuclease VII (xseA)), AF134570 (Xenopus laevis exonuclease Exol (EXO1)), AF084974 (Homo sapiens exonuclease I (EXO1)), AF030933 (Homo sapiens

exonuclease homolog RAD1 (RAD1)), AF034258 (*Caenorhabditis elegans* exonuclease III homolog), AH006967 (*Homo sapiens* exonuclease I (EXO1) gene), AF091740 (*Homo sapiens* exonuclease 1a (EXO1a), 5174 (*Schizosaccharomyces pombe* exonuclease I (exo1)), AF084514 (*Mus musculus* DNA repair exonuclease (Rec1)), AF084513 (*Homo sapiens* DNA repair exonuclease (REC1)), AF084512 (*Homo sapiens* DNA repair exonuclease (REC1)), AF060479 (*Homo sapiens* exonuclease I (EXO1), U76424 (*Lactococcus lactis*), U57401 (*Choristoneura fumiferana* alkaline exonuclease), U58147 (*Haemophilus ducreyi*), U86134 (*Saccharomyces cerevisiae* exonuclease 1 (EXO1), U57963 (*Erwinia chrysanthemi* single-stranded DNA exonuclease (recJ) gene), M22592 (*E.coli* xth gene encoding exonuclease III), J02641 (*E.coli* sbcB gene encoding exonuclease I), L23927 (*Escherichia coli* exonuclease VIII (recE)

Preferably, exonucleases that specifically cleave double-stranded nucleic acids, but not single-stranded nucleic acids, are used in the present methods. Also preferably, nuclease BAL-31, exonuclease III, Mung Bean exonuclease or Lambda exonuclease is used.

#### G. LABELLING OF MUTANT DNA REPAIR ENZYMES

Conjugates, such as fusion proteins and chemical conjugates, of the mutant DNA repair enzyme with a protein or peptide fragment (or plurality thereof) that functions, for example, to facilitate affinity isolation or purification of the mutant enzyme, attachment of the mutant enzyme to a surface, or detection of the mutant enzyme are provided. The conjugates can be produced by chemical conjugation, such as via thiol linkages, but are preferably produced by recombinant means as fusion proteins. In the fusion protein, the peptide or fragment thereof is linked to either the N-terminus or C-terminus of the mutant enzyme. In chemical conjugates the peptide or fragment thereof may be linked anywhere that conjugation can be effected, and there may be a plurality of such peptides or fragments linked to a single mutant enzyme or to a plurality thereof.

**1. Conjugation**

Conjugation can be effected by any method known to those of skill in the art. As described below, conjugation can be effected by chemical means, through covalent, ionic or any other suitable linkage.

**5 a. Fusion proteins**

Fusion proteins are provided herein. A fusion protein contains: a) one or a plurality of mutant DNA repair enzymes and b) at least one protein or peptide fragment that facilitates, for example: i) affinity isolation or purification of the fusion protein; ii) attachment of the fusion 10 protein to a surface; or iii) detection of the fusion protein, or any combination thereof.

The facilitating agent is selected to perform the desired purpose, such as (i) - (iii), and is linked a mutant DNA repair enzyme such that the resulting conjugate retains the mutant DNA repair enzyme property and 15 also processes the property(ies) of the facilitating agent. For example, the facilitating agent can be a protein or peptide fragment, such as a protein binding peptide, including but not limited to an epitope tag or an IgG binding protein, a nucleotide binding protein, such as a DNA or RNA binding protein, a lipid binding protein, a polysaccharide binding protein, 20 and a metal binding protein or fragments thereof that possess the requisite desired facilitating activity.

Such facilitating agents can be designed, screened or selected according to the methods known in the art. The screening or selection process begins, for example, with nucleic acid encoding a particular 25 protein or peptide to be used in the fusion protein, and screened or selected for its specific binding partner. Alternatively, the screening or selection process can start with a specific molecule that can be used in the subsequent isolation/purification, attachment or detection, and screen or select for a particular protein or peptide sequence to be used in the 30 fusion protein that can specifically bind to the pre-selected molecule.

The conventional technique of random screening of natural

products can be used in screening and selecting a protein or peptide sequence and its specific binding partner. In addition, numerous strategies can be used for preparing proteins having new binding specificities. These new approaches generally involve the synthetic 5 production of large numbers of random molecules followed by some selection procedure to identify the molecule of interest. For example, epitope libraries have been developed using random polypeptides displayed on the surface of filamentous phage particles. The library is made by synthesizing a repertoire of random oligonucleotides to generate 10 all combinations, followed by their insertion into a phage vector. Each of the sequences is separately cloned and expressed in phage, and the relevant expressed peptide can be selected by finding those phage that bind to the particular target. The phages recovered in this way can be amplified and the selection repeated. The sequence of the peptide is 15 decoded by sequencing the DNA (*See e.g.*, Cwirla et al., *Proc. Natl. Acad. Sci., USA*, 87:6378-6382 (1990); Scott et al., *Science*, 249:386-390 (1990); and Devlin et al., *Science*, 249:404-406 (1990)).

Another approach involves large arrays of peptides that are synthesized in parallel and screened with acceptor molecules labelled with 20 fluorescent or other reporter groups. The sequence of any effective peptide can be decoded from its address in the array (*See e.g.*, Geysen et al., *Proc. Natl. Acad. Sci., USA*, 81:3998-4002 (1984); Maeji et al., *J. Immunol. Met.*, 146:83-90 (1992); and Fodor et al., *Science*, 251:767-775 (1991)).

25 Combinatorial approaches can also be employed. For example, in one exemplary approach, combinatorial libraries of peptides are synthesized on resin beads such that each resin bead contains about 20 pmoles of the same peptide. The beads are screened with labeled acceptor molecules and those with bound acceptor are searched for by 30 visual inspection, physically removed, and the peptide identified by direct sequence analysis (Lam et al., *Nature*, 354:82-84 (1991)). Another

useful combinatorial method for identification of peptides of desired activity is that of Houghten et al. (see, e.g., *Nature*, 354:84-86 (1991)). For hexapeptides of the 20 natural amino acids, 400 separate libraries are synthesized, each with the first two amino acids fixed and the remaining 5 four positions occupied by all possible combinations. An assay, based on competition for binding or other activity, is then used to find the library with an active peptide. Twenty new libraries are then synthesized and assayed to determine the effective amino acid in the third position, and the process is reiterated in this fashion until the active hexapeptide is 10 defined.

b. **Chemical conjugation**

To effect chemical conjugation herein, the targeting agent is linked via one or more selected linkers or directly to the targeted agent. Chemical conjugation must be used if the targeted agent is other than a 15 peptide or protein, such as a nucleic acid or a non-peptide drug. Any means known to those of skill in the art for chemically conjugating selected moieties may be used.

1) **Heterobifunctional cross-linking reagents**

Numerous heterobifunctional cross-linking reagents that are used to 20 form covalent bonds between amino groups and thiol groups and to introduce thiol groups into proteins, are known to those of skill in this art (see, e.g., the PIERCE CATALOG, ImmunoTechnology Catalog & Handbook, 1992-1993, which describes the preparation of and use of such reagents and provides a commercial source for such reagents; see, 25 also, e.g., Cumber et al. (1992) *Bioconjugate Chem.* 3:397-401; Thorpe et al. (1987) *Cancer Res.* 47:5924-5931; Gordon et al. (1987) *Proc. Natl. Acad. Sci.* 84:308-312; Walden et al. (1986) *J. Mol. Cell Immunol.* 2:191-197; Carlsson et al. (1978) *Biochem. J.* 173: 723-737; Mahan et al. (1987) *Anal. Biochem.* 162:163-170; Wawryznaczak et al. (1992) *Br. 30 J. Cancer* 66:361-366; Fattom et al. (1992) *Infection & Immun.* 60:584-589). These reagents may be used to form covalent bonds between the

mutant analyte binding enzyme and the facilitating agent. These reagents include, but are not limited to: N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP; disulfide linker); sulfosuccinimidyl 6-[3-(2-pyridyldithio)propionamido]hexanoate (sulfo-LC-SPDP);

5 succinimidyl oxycarbonyl- $\alpha$ -methyl benzyl thiosulfate (SMBT, hindered disulfate linker); succinimidyl 6-[3-(2-pyridyldithio) propionamido]-hexanoate (LC-SPDP); sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC); succinimidyl 3-(2-pyridyldithio)-butyrate (SPDB; hindered disulfide bond linker); sulfosuccinimidyl 2-(7-

10 azido-4-methylcoumarin-3-acetamide) ethyl-1,3'-dithiopropionate (SAED); sulfo-succinimidyl 7-azido-4-methylcoumarin-3-acetate (SAMCA); sulfosuccinimidyl 6-[alpha-methyl-alpha-(2-pyridyldithio)toluamido]-hexanoate (sulfo-LC-SMPT); 1,4-di-[3'-(2'-pyridyldithio)propionamido]butane (DPDPB); 4-succinimidyl oxycarbonyl- $\alpha$ -methyl- $\alpha$ -(2-

15 pyridylthio)toluene (SMPT, hindered disulfate linker); sulfosuccinimidyl 6[ $\alpha$ -methyl- $\alpha$ -(2-pyridyldithio)toluamido]hexanoate (sulfo-LC-SMPT); m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS); m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBS); N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB; thioether linker); sulfosuccinimidyl(4-

20 iodoacetyl)amino benzoate (sulfo-SIAB); succinimidyl 4-( $\rho$ -maleimidophenyl)butyrate (SMPB); sulfosuccinimidyl 4-( $\rho$ -maleimidophenyl)butyrate (sulfo-SMPB); azidobenzoyl hydrazide (ABH).

Other heterobifunctional cleavable cross-linkers include, N-succinimidyl (4-iodoacetyl)-aminobenzoate; sulfosuccinimidyl (4-iodoacetyl)-aminobenzoate; 4-succinimidyl-oxycarbonyl- $\alpha$ -(2-pyridyldithio)-toluene; sulfosuccinimidyl-6-[ $\alpha$ -methyl- $\alpha$ -(pyridyldithiol)-toluamido]hexanoate; N-succinimidyl-3-(2-pyridyldithio)-propionate; succinimidyl 6[3-(2-pyridyldithio)-propionamido]hexanoate; sulfosuccinimidyl 6[3-(2-pyridyldithio)-propionamido]hexanoate; 3-(2-pyridyldithio)-propionyl hydrazide, Ellman's reagent, dichlorotriazinic acid, S-(2-thiopyridyl)-L-cysteine. Further exemplary bifunctional linking compounds are disclosed

in U.S. Patent Nos. 5,349,066, 5,618,528, 4,569,789, 4,952,394, and 5,137,877.

## 2) Exemplary Linkers

Any linker known to those of skill in the art for preparation of conjugates may be used herein. These linkers are typically used in the preparation of chemical conjugates; peptide linkers may be incorporated into fusion proteins.

Linkers can be any moiety suitable to associate the mutant DNA repair enzyme and the facilitating agent. Such linkers and linkages include, but are not limited to, peptidic linkages, amino acid and peptide linkages, typically containing between one and about 60 amino acids, more generally between about 10 and 30 amino acids, chemical linkers, such as heterobifunctional cleavable cross-linkers, including but are not limited to, N-succinimidyl (4-iodoacetyl)-aminobenzoate, sulfosuccinimidyl (4-iodoacetyl)-aminobenzoate, 4-succinimidyl-oxycarbonyl-a-(2-pyridyldithio)toluene, sulfosuccinimidyl-6-[a-methyl-a-(pyridyldithio)-toluamido] hexanoate, N-succinimidyl-3-(2-pyridyldithio) - propionate, succinimidyl 6[3(-2-pyridyldithio)-propionamido] hexanoate, sulfosuccinimidyl 6[3(-2-pyridyldithio)-propionamido] hexanoate, 3-(2-pyridyldithio)-propionyl hydrazide, Ellman's reagent, dichlorotriazinic acid, and S-(2-thiopyridyl)-L-cysteine. Other linkers include, but are not limited to peptides and other moieties that reduce stearic hindrance between the mutant analyte binding enzyme and the facilitating agent, intracellular enzyme substrates, linkers that increase the flexibility of the conjugate, linkers that increase the solubility of the conjugate, linkers that increase the serum stability of the conjugate, photocleavable linkers and acid cleavable linkers.

Other exemplary linkers and linkages that are suitable for chemically linked conjugates include, but are not limited to, disulfide bonds, thioether bonds, hindered disulfide bonds, and covalent bonds between free reactive groups, such as amine and thiol groups. These 5 bonds are produced using heterobifunctional reagents to produce reactive thiol groups on one or both of the polypeptides and then reacting the thiol groups on one polypeptide with reactive thiol groups or amine groups to which reactive maleimido groups or thiol groups can be attached on the other. Other linkers include, acid cleavable linkers, such as 10 bismaleimideethoxy propane, acid labile-transferrin conjugates and adipic acid dihydrazide, that would be cleaved in more acidic intracellular compartments; cross linkers that are cleaved upon exposure to UV or visible light and linkers, such as the various domains, such as C<sub>H</sub>1, C<sub>H</sub>2, and C<sub>H</sub>3, from the constant region of human IgG, (see, Batra *et al.* (1993) 15 *Molecular Immunol.* 30:379-386). In some embodiments, several linkers may be included in order to take advantage of desired properties of each linker.

Chemical linkers and peptide linkers may be inserted by covalently coupling the linker to the mutant DNA repair enzyme and the facilitating 20 agent. The heterobifunctional agents, described below, may be used to effect such covalent coupling. Peptide linkers may also be linked by expressing DNA encoding the linker and TA, linker and targeted agent, or linker, targeted agent and TA as a fusion protein. Flexible linkers and linkers that increase solubility of the conjugates are contemplated for use, 25 either alone or with other linkers are also contemplated herein.

a) **Acid cleavable, photocleavable and heat sensitive linkers**

Acid cleavable linkers, photocleavable and heat sensitive linkers may also be used, particularly where it may be necessary to cleave the 30 targeted agent to permit it to be more readily accessible to reaction. Acid cleavable linkers include, but are not limited to, bismaleimideethoxy

propane; and adipic acid dihydrazide linkers (see, e.g., Fattom *et al.* (1992) *Infection & Immun.* 60:584-589) and acid labile transferrin conjugates that contain a sufficient portion of transferrin to permit entry into the intracellular transferrin cycling pathway (see, e.g., Welhöner *et al.* (1991) *J. Biol. Chem.* 266:4309-4314).

Photocleavable linkers are linkers that are cleaved upon exposure to light (see, e.g., Goldmacher *et al.* (1992) *Bioconj. Chem.* 3:104-107, which linkers are herein incorporated by reference), thereby releasing the targeted agent upon exposure to light. Photocleavable linkers that are cleaved upon exposure to light are known (see, e.g., Hazum *et al.* (1981) in *Pept., Proc. Eur. Pept. Symp.*, 16th, Brunfeldt, K (Ed), pp. 105-110, which describes the use of a nitrobenzyl group as a photocleavable protective group for cysteine; Yen *et al.* (1989) *Makromol. Chem.* 190:69-82, which describes water soluble photocleavable copolymers, including hydroxypropylmethacrylamide copolymer, glycine copolymer, fluorescein copolymer and methylrhodamine copolymer; Goldmacher *et al.* (1992) *Bioconj. Chem.* 3:104-107, which describes a cross-linker and reagent that undergoes photolytic degradation upon exposure to near UV light (350 nm); and Senter *et al.* (1985) *Photochem. Photobiol.* 42:231-237, which describes nitrobenzyloxycarbonyl chloride cross linking reagents that produce photocleavable linkages), thereby releasing the targeted agent upon exposure to light. Such linkers would have particular use in treating dermatological or ophthalmic conditions that can be exposed to light using fiber optics. After administration of the conjugate, the eye or skin or other body part can be exposed to light, resulting in release of the targeted moiety from the conjugate. Such photocleavable linkers are useful in connection with diagnostic protocols in which it is desirable to remove the targeting agent to permit rapid clearance from the body of the animal.

**b) Other linkers for chemical conjugation**

Other linkers, include trityl linkers, particularly, derivatized trityl groups to generate a genus of conjugates that provide for release of therapeutic agents at various degrees of acidity or alkalinity.

5 The flexibility thus afforded by the ability to preselect the pH range at which the therapeutic agent will be released allows selection of a linker based on the known physiological differences between tissues in need of delivery of a therapeutic agent (see, e.g., U.S. Patent No. 5,612,474). For example, the acidity of tumor tissues appears to be lower than that of  
10 normal tissues.

**c) Peptide linkers**

The linker moieties can be peptides. Peptide linkers can be employed in fusion proteins and also in chemically linked conjugates. The peptide typically has from about 2 to about 60 amino acid residues, for  
15 example from about 5 to about 40, or from about 10 to about 30 amino acid residues. The length selected will depend upon factors, such as the use for which the linker is included.

The proteinaceous ligand binds with specificity to a receptor(s) on one or more of the target cell(s) and is taken up by the target cell(s). In  
20 order to facilitate passage of the chimeric ligand-toxin into the target cell, it is presently preferred that the size of the chimeric ligand-toxin be no larger than can be taken up by the target cell of interest. Generally, the size of the chimeric ligand-toxin will depend upon its composition. In the case where the chimeric ligand toxin contains a chemical linker and a  
25 chemical toxin (i.e., rather than proteinaceous one), the size of the ligand toxin is generally smaller than when the chimeric ligand-toxin is a fusion protein. Peptidic linkers can conveniently be encoded by nucleic acid and

incorporated in fusion proteins upon expression in a host cell, such as *E. coli*.

Peptide linkers are advantageous when the facilitating agent is proteinaceous. For example, the linker moiety can be a flexible spacer 5 amino acid sequence, such as those known in single-chain antibody research. Examples of such known linker moieties include, but are not limited to, peptides, such as  $(\text{Gly}_m\text{Ser})_n$  and  $(\text{Ser}_m\text{Gly})_n$ , in which n is 1 to 6, preferably 1 to 4, more preferably 2 to 4, and m is 1 to 6, preferably 1 to 4, more preferably 2 to 4, enzyme cleavable linkers and others.

10 Additional linking moieties are described, for example, in Huston *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 85:5879-5883, 1988; Whitlow, M., *et al.*, *Protein Engineering* 6:989-995, 1993; Newton *et al.*, *Biochemistry* 35:545-553, 1996; A. J. Cumber *et al.*, *Bioconj. Chem.* 3:397-401, 1992; Ladurner *et al.*, *J. Mol. Biol.* 273:330-337, 1997; and U.S. Patent. 15 No. 4,894,443. In some embodiments, several linkers may be included in order to take advantage of desired properties of each linker.

## 2. Selection of facilitating agents

Any agent that facilitates detection, immobilization, or purification of the conjugate is contemplated for use herein. For chemical conjugates 20 any moiety that has such properties is contemplated; for fusion proteins, the facilitating agent is a protein, peptide or fragment thereof that is sufficient to effect the facilitating activity.

### a. Protein binding moieties

The conjugate contains a protein binding moiety, particularly a 25 protein binding protein, peptide or effective fragment thereof. Its specific binding partner can be proteins or peptides generally, a set of proteins or peptides or mixtures thereof, or a particular protein or peptide. Any protein-protein interaction pair known to those of skill in the art is contemplated. For example, the protein-protein interaction pair can be 30 enzyme/protein or peptide substrate, antibody/protein or peptide antigen, receptor/protein or peptide ligand, etc. Any protein-protein interaction

pair can be designed, screened or selected according to the methods known in the art (See generally, *Current Protocols in Molecular Biology* (1998) § 20, John Wiley & Sons, Inc.). Examples of such methods for identifying protein-protein interactions include the interaction trap/two-hybrid system and the phage-based expression cloning.

5           1)     Interaction trap/two-hybrid system

Interacting proteins can be identified by a selection or screen in which proteins that specifically interact with a target protein of interest are isolated from a library. One particular approach to detect interacting 10 proteins is the two-hybrid system or interaction trap (See generally, *Current Protocols in Molecular Biology* (1998) § 20.1.-20.2., John Wiley & Sons, Inc.), which uses yeast as a "test tube" and transcriptional activation of a reporter system to identify associating proteins.

In the two-hybrid system, a yeast vector such as the plasmid 15 pEG202 or a related vector can be used to express the probe or "bait" protein as a fusion to the heterologous DNA-binding protein LexA. Many proteins, including transcription factors, kinases, and phosphatases, can be used as bait proteins. The major requirements for the bait protein are that it should not be actively excluded from the yeast nucleus, and it 20 should not possess an intrinsic ability to strongly activate transcription. The plasmid expressing the LexA-fused bait protein can be used to transform yeast possessing a dual reporter system responsive to transcriptional activation through the LexA operator.

In one such example, the yeast strain EGY48 containing the 25 reporter plasmid pSH18-34 can be used. In this case, binding sites for LexA are located upstream of two reporter genes. In the EGY48 strain, the upstream activating sequences of the chromosomal *LEU2* gene, which is required in the biosynthetic pathway for leucine (Leu), are replaced with LexA operators (DNA binding sites). PSH18-34 contains a 30 LexA operator-*lacZ* fusion gene. These two reporters allow selection for transcriptional activation by permitting selection for viability when cells

are plated on medium lacking Leu, and discrimination based on color when the yeast is grown on medium containing Xgal.

The EGY48/PSH18-34 transformed with a bait is first characterized for its ability to express protein, growth on medium lacking Leu, and for 5 the level of transcriptional activation of *lacZ*. A number of alternative strains, plasmids, and strategies can be employed if a bait proves to have an unacceptably high level of background transcriptional activation.

In an interactor hunt, the strain EGY48/PSH18-34 containing the bait expression plasmid is transformed, preferably along with carrier DNA, 10 with a conditionally expressed library made in a suitable vector such as the vector pJG4-5. This library uses the inducible yeast GAL1 promoter to express proteins as fusions to an acidic domain ("acid blob") that functions as a portable transcriptional activation motif (act) and to other useful moieties. Expression of library-encoded proteins is induced by 15 plating transformants on medium containing galactose (Gal), so yeast cells containing library proteins that do not interact specifically with the bait protein will fail to grow in the absence of Leu. Yeast cells containing library proteins that interact with the bait protein will form colonies within 2 to 5 days, and the colonies will turn blue when the cells are streaked 20 on medium containing Xgal. The DNA from interaction trap positive colonies can be analyzed by polymerase chain reaction (PCR) to streamline screening and detect redundant clones in cases where many positives are obtained in screening. The plasmids can be isolated and characterized by a series of tests to confirm specificity of the interaction 25 with the initial bait protein.

An alternative way of conducting an interactor hunt is to mate a strain that expresses the bait protein with a strain that has been pretransformed with the library DNA, and screen the resulting diploid cells for interactors (Bendixen et al., *Nucl. Acids. Res.*, 22:1778-1779 (1994); 30 and Finley and Brent, *Proc. Natl. Sci. U.S.A.*, 91:12980-12984 (1994)). This "interaction mating" approach can be used for any interactor hunt,

and is particularly useful in three special cases. The first case is when more than one bait will be used to screen a single library. Interaction mating allows several interactor hunts with different baits to be conducted using a single high-efficiency yeast transformation with library DNA. This can be a considerable savings, since the library transformation is one of the most challenging tasks in an interactor hunt. The second case is when a constitutively expressed bait interferes with yeast viability. For such baits, performing a hunt by interaction mating avoids the difficulty associated with achieving a high-efficiency library transformation of a strain expressing a toxic bait. Moreover, the actual selection for interactors will be conducted in diploid yeast, which are more vigorous than haploid yeast and can better tolerate expression of toxic proteins. The third case is when a bait cannot be used in a traditional interactor hunt using haploid yeast stains because it activates transcription of even the least sensitive reporters. In diploids the reporters are less sensitive to transcription activation than they are in haploids. Thus, the interaction mating hunt provides an additional method to reduce background from transactivating baits.

The interaction trap/two-hybrid system and the identified protein-protein interaction pairs have been successfully used (see, e.g., Bartel et al., Using the two-hybrid system to detect protein-protein interactions, *In Cellular Interactions in Development: A Practical Approach*, (D.A. Hartley, ed.) pp. 153-179, Oxford University Press, Oxford (1993); Bartel et al., A protein linkage map of *Escherichia coli* bacteriophage T7, *Nature Genet.*, 12:72-77 (1996); Bendixen et al., A yeast mating-selection scheme for detection of protein-protein interactions, *Nucl. Acids. Res.*, 22:1778-1779 (1994); Breeden and Nasmyth, Regulation of the yeast HO gene., *Cold spring Harbor Symp. Quant. Biol.*, 50:643-650 (1985); Brent and Ptashne, A bacterial repressor protein or a yeast transcriptional terminator can block upstream activation of a yeast gene, *Nature*, 312:612-615 (1984); Brent et al., A eukaryotic transcriptional activator bearing the

DNA specificity of a prokaryotic repressor, *Cell*, 43:729-736 (1985); Chien et al., The two-hybrid system: A method to identify and clone genes for proteins that interact with a protein of interest, *Proc. Natl. Acad. Sci. U.S.A.*, 88:9578-9582 (1991); Chiu et al., RAPT1, a 5 mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex, *Proc. Natl. Acad. Sci. U.S.A.*, 91:12574-12578 (1994); Colas et al., Genetic selection of peptide aptamers that recognize and inhibit cyclin-dependent kinase 2., *Nature*, 380:548-550 (1996); Durfee et al., The retinoblastoma protein associates with the protein phosphatase type 10 catalytic subunit, *Genes & Dev.*, 7:555-569 (1993); Estojak et al., Correlation of two-hybrid affinity data with in vitro measurements, *Mol. Cell. Biol.*, 15:5820-5829 (1995); Fearon et al., Karyoplasmic interaction selection strategy: A general strategy to detect protein-protein interaction in mammalian cells, *Proc. Natl. Acad. Sci. U.S.A.*, 89:7958-7962 (1992); 15 Fields and Song, A novel genetic system to detect protein-protein interaction, *Nature*, 340:245-246 (1989); Finley and Brent, Interaction mating reveals binary and ternary connections between *Drosophila* cell cycle regulators, *Proc. Natl. Acad. Sci. U.S.A.*, 91:12980-12984 (1994); Gietz et al., Improved method for high-efficiency transformation of intact yeast 20 cells, *Nucl. Acids. Res.*, 20:1425 (1992); Golemis and Brent, Fused protein domains inhibit DNA binding by LexA, *Mol. Cell Biol.*, 12:3006-3014 (1992); Gyuris et al., Cdi1, a human G1 and S-phase protein phosphatase that associates with Cdk1, *Cell*, 75:791-803 (1993); Kaiser et al., A., Methods in Yeast Genetics, a Cold Spring Harbor Laboratory 25 Course Manual, pp. 135-136. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1994); Kolonin and Finley, Jr., Targeting cyclin-dependent kinases in *Drosophila* with peptide aptamers, *Proc. Natl. Acad. Sci. U.S.A.*, In press (1998); Licitra and Liu, A three-hybrid system for detecting small ligand-protein receptor interactions, *Proc. Natl. Acad. Sci. 30 U.S.A.*, 93:12817-12821 (1996); Ma and Ptashne, A new class of yeast transcriptional activators, *Cell*, 51:113-119 (1987); Ma and Ptashne,

Converting an eukaryotic transcriptional inhibitor into an activator, *Cell*, 55:443-446 (1988); Osborne et al., The yeast tribrid system: Genetic detection of transphosphorylated ITAM-SH2 interactions, *Bio/Technology*, 13:1474-1478 (1995); Ruden et al., Generating yeast transcriptional activators containing no yeast protein sequences, *Nature*, 350:426-430 (1991); Samson et al., Gene activation and DNA binding by *Drosophila* Ubx and abd-A proteins, *Cell*, 57:1045-1052 (1989); Stagljar et al., Use of the two-hybrid system and random sonicated DNA to identify the interaction domain of a protein, *BioTechniques*, 21:430-432 (1996); Vasavada et al., A contingent replication assay for the detection of protein-protein interactions in animal cells, *Proc. Nat. Acad. Sci. U.S.A.*, 88:10686-10690 (1991); Vojtex et al., Mammalian Ras interacts directly with the serine/threonine kinase Raf, *Cell*, 74:205-214 (1993); Watson et al., Vectors encoding alternative antibiotic resistance for use in the yeast two-hybrid system, *BioTechniques*, 21:255-259 (1996); West et al., *Saccharomyces cerevisiae* GAL10 divergent promoter region: Location and function of the upstream activator sequence UASG, *Mol. Cell Biol.*, 4:2467-2478 (1984); and Yang et al., Protein-peptide interactions analyzed with the yeast two-hybrid system, *Nucl. Acids Res.*, 23:1152-1156 (1995)) and can be used in the present system.

2) Phage-based expression cloning

Interaction cloning (also known as expression cloning) is a technique to identify and clone genes that encode proteins that interact with a protein of interest, or "bait" protein. Phage-based interaction cloning requires a gene encoding the bait protein and an appropriate expression library constructed in a bacteriophage expression vector, such as λgt11 (See generally, *Current Protocols in Molecular Biology* (1998) § 20.3, John Wiley & Sons, Inc.). The gene encoding the bait protein is used to produce recombinant fusion protein in *E. coli*. The cDNA is radioactively labeled with <sup>32</sup>P. A recognition site for a protein kinase such as the cyclic adenosine 3',5'-phosphate (cAMP)--dependent protein

kinase (Protein kinase A; PKA) is introduced into the recombinant fusion protein to allow its enzymatic phosphorylation by the kinase and [ $\lambda$ -<sup>32</sup>P]ATP.

In one example, the procedure involves a fusion protein containing 5 bait protein and glutathione-S-transferase (GST) with a PKA site at the junction between them. The labeled protein is subsequently used as a probe to screen a  $\lambda$  bacteriophage-derived cDNA expression library, which expresses  $\beta$ -galactosidase fusion proteins that contain in-frame gene fusions. The phages lyse cells, form plaques, and release fusion proteins 10 that are adsorbed onto nitrocellulose membrane filters. The filters are blocked with excess nonspecific protein to eliminate nonspecific binding and probed with the radiolabeled bait protein. This procedure leads directly to the isolation of genes encoding the interacting protein, bypassing the need for purification and microsequencing or for antibody 15 production.

The phage-based interaction cloning system and the identified protein-protein interaction pairs have been successfully employed (Blanar et al., Interaction cloning: Identification of a helix-loop-helix zipper protein that interacts with c-Fos, *Science*, 256:1014-1018 (1992); Carr and 20 Scott, Blotting and band-shifting: Techniques for studying protein-protein interactions, *Trends Biochem. Sci.*, 17:246-249 (1992); Chapline et al., Interaction cloning of protein kinase C substrates, *J. Biol. Chem.*, 268:6858-6861 (1993); Hoeffler et al., Identification of multiple nuclear factors that interact with cyclic AMP response element-binding protein 25 and activation transcription factor-2 by protein interactions, *Mol. Endocrinol.*, 5:256-266 (1991); Kaelin et al., Expression cloning of a cDNA encoding a retinoblastoma-binding protein with E2F-like properties, *Cell*, 70:351-364 (1992); Lester et al., Cloning and characterization of a novel A-kinase anchoring protein: AKAP220, association with testicular 30 peroxisomes, *J. Biol. Chem.*, 271:9460-9465 (1996); Lowenstein et al., The SH2 and SH2 domain-containing protein GRB2 links receptor tyrosine

kinase to ras signaling, *Cell*, 70:431-442 (1992); Margolis et al., High-efficiency expression/cloning of epidermal growth factor-receptor-binding proteins with *src* homology 2 domains, *Proc. Natl. Acad. Sci. U.S.A.*, 89:8894-8898 (1992); Skolnik et al., Cloning of P13 kinase-associated p85 utilizing a novel method of expression/cloning of target proteins for receptor tyrosine kinases, *Cell*, 65:83-90 (1991); and Stone et al., Interaction of a protein phosphatase with an *Arabidopsis* serine-threonine receptor kinase, *Science*, 266:793-795 (1994)) and can be used in the present system.

10                   3)     Detection of protein-protein interactions

Surface plasmon resonance (SPR) can be used to verify the protein-protein interactions identified by other systems such as the interaction trap/two-hybrid system and the phage-based expression cloning systems (See generally, *Current Protocols in Molecular Biology* (1998) § 20.4, 15 John Wiley & Sons, Inc.). This is an *in vitro* technique based on an optical phenomenon, called SPR, that can simultaneously detect interactions between unmodified proteins and directly measure kinetic parameters of the interaction.

SPR devices are commercially available. The BIACore instrument 20 (BIACore) is presently preferred herein. This instrument contains sensing optics, an automated sample delivery system, and a computer for instrument control, data collection, and data processing. Experiments are performed on disposable chips. In practice, a ligand protein is immobilized on the chip while buffer continuously flows over the surface. 25 The sensing apparatus monitors changes in the angle of minimum reflectance from the interface that result when a target protein associates with the ligand protein. Molecular interactions can be directly visualized (on the computer monitor) in real time as the optical response is plotted against time. This response is measured in resonance units (RUs, where 30 1000 RUs = 1 ng protein/mm<sup>2</sup>).

The SPR system has been successfully used (see, e.g.,

BioSupplyNet Source Book, BioSupplyNet, Plainview, N.Y., and Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1999); Feng et al., Functional binding between G $\beta$  and the LIM domain of Ste5 is required to activate the MEKK Ste11, *Cur. Biol.*, 8:267-278 (1998); Field et al., Purification of RAS-responsive adenylyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method, *Mol. Cell. Biol.*, 8:2159-2165 (1988); Phizicky and Fields, Protein-protein interactions: Methods for detection and analysis, *Microbiol. Rev.*, 59:94-123 (1995); Tyers et al., Comparison of the *Saccharomyces cerevisiae* G1 cyclins: Cln3 may be an upstream activator of Cln1, Cln2, and other cyclins, *EMBO J.*, 11:1773-1784 (1993) and the identified protein-protein interaction pairs can be used in the present system.

b. Epitope tags

The facilitating agent can be any moiety, particularly a protein, peptide or effective fragment thereof that is specifically recognized by an antibody. In these embodiments, the conjugate contains an epitope tag that is specifically recognized by a set of antibodies or by a particular antibody. Any epitope/antibody pair can be used in the present system (See generally, *Current Protocols in Molecular Biology* (1998) 10.15, John Wiley & Sons, Inc.). The following Table 3 provides exemplary epitope tags and illustrates certain properties of several commonly used epitope tag systems.

**Table 3. Exemplary epitope tag systems**

	Epitope	Peptide	SEQ ID	Antibody	Reference
25	FLAG	AspTyrLysAspAspAspLys	1	4E11	Prickett <sup>1</sup>
	HA	TyrProTyrAspValProAspTyrAla	2	12Ca5	Xie <sup>2</sup>
	HA1	CysGlnAspLeuProGlyAsnAspAsnSerThr	3	mouse MAb	Nagelkerken <sup>3</sup>
	c-Myc	GluGlnLysLeuIleSerGluGluAspLeu	4	9E10	Xie <sup>2</sup>
30	6-His	HisHisHisHisHis	5	BAbCO <sup>*</sup>	
	AU1	AspThrTyrArgTyrIle	6	BAbCO	
	EE	GluTyrMetProMetGlu	7	anti-EE	Tolbert <sup>4</sup>

Epitope	Peptide	SEQ ID	Antibody	Reference
T7	AlaSerMetThrGlyGlyGlnGlnMetGlyArg	8	Invitrogen	Chen <sup>5</sup> Tseng <sup>6</sup>
4A6	SerPheProGlnPheLysProGlnGlutle	9	4A6	Rudiger <sup>7</sup>
$\epsilon$	LysGlyPheSerTyrPheGlyGluAspLeuMetPro	10	anti-PKCe	Olah <sup>8</sup>
B	GlnTyrProAlaLeuThr	11	D11, F10	Wang <sup>9</sup>
gE	GlnArgGlnTyrGlyAspValPheLysGlyAsp	12	3B3	Grose <sup>10</sup>
Ty1	GluValHisThrAsnGlnAspProLeuAsp	13	BB2, TYG5	Bastin <sup>11</sup>

1. Prickett et al., *BioTechniques*, 7(6):580-584 (1989)  
 2. Xie et al., *Endocrinology*, 139(11):4563-4567 (1998)  
 10 3. Nagelkerke et al., *Electrophoresis*, 18:2694-2698 (1997)  
 4. Tolbert and Lameh, *J. Neurochem.*, 70:113-119 (1998)  
 5. Chen and Katz, *BioTechniques*, 25(1):22-24 (1998)  
 6. Tseng and Verma, *Gene*, 169:287-288 (1996)  
 7. Rudiger et al., *BioTechniques*, 23(1):96-97 (1997)  
 15 8. Olah et al., *Biochem.*, 221:94-102 (1994)  
 9. Wang et al., *Gene*, 169(1):53-58 (1996)  
 10. Grose, U.S. Patent No. 5,710,248  
 11. Bastin et al., *Mol. Biochem. Parasitology*, 77:235-239 (1996)  
 Invitrogen, Sigma, Santa Cruz Biotech

20 For example, in one embodiment, the selected epitope tag is the 6-His tag. Vectors for constructing a fusion protein containing the 6-His tag and reagents for isolating or purifying such fusion proteins are commercially available. For example, the Poly-His gene fusion vector from Invitrogen, Inc. (Carlsbad, CA) includes the following features: 1) 25 high-level regulated transcription for the *trc* promotor; 2) enhanced translation efficiency of eukaryotic genes in *E.coli*; 3) the *LacO* operator and the *LacI<sup>r</sup>* repressor gene for transcriptional regulation in any *E. coli* system; N-terminal Xpress epitope for easy detection with an Anti-Xpress antibody; and 4) enterokinase cleaving site for removal of the fusion tag.

30 The fusion protein can be purified by nickel-chelating agarose resin, and the purified fusion protein can be coated onto a microtiter plate pre-coated with nickel (e.g., Reacti-Binding meta chelate polystyrene plates, Pierce) for diagnostic usage.

In addition, the fusion protein containing the 6-His tag can be isolated or purified using the His MicroSpin Purification Module or HisTrap Kit from Amersham Pharmacia Biotech, Inc. The His MicroSpin Purification Module provides fifty MicroSpin columns prepacked with 5 nickel-charged Chelating Sepharose Fast Flow. The module enables the simple and rapid screening of large numbers of small-scale bacterial lysates for the analysis of putative clones and optimization of expression and purification conditions. Each column contains 50  $\mu$ l bed volume, enough to purify > 100  $\mu$ g his-tagged fusion protein, from up to 400  $\mu$ l 10 of His-tagged fusion protein sample, e.g., crude lysate and purification intermediates. The HisTrap Kit is designed for rapid, mild affinity purification of histidine-tagged fusion proteins in a single step. The high dynamic capacity of HiTrap Chelating enables milligrams of protein to be purified in less than 15 minutes at flow rates of up to 240 column 15 volumes per hour. The high capacity is maintained after repeated use ensuring cost-effective, reproducible purifications. The Kit includes three HiTrap Chelating columns and buffer concentrates to perform F10-12 purifications with a syringe. The anti-His antibody from Amersham Pharmacia Biotech, Inc. is an IgG<sub>2</sub> subclass of monoclonal antibody 20 directed against 6 Histidine residues. The antibody is unconjugated to offer the flexibility of detection with a secondary antibody conjugated with either horseradish peroxidase or alkaline phosphatase. The antibody provides high sensitivity with low background.

Further examples of epitope tagging can be found in Kolodziej and 25 Young, *Epitope tagging and protein surveillance, Methods Enzymol.*, 194:508-519 (1991). Methods for preparing and using other such tags and other such tags similarly can be used in the methods and products provided herein.

c. IgG binding proteins

In other embodiments, the conjugate contains an IgG binding protein, which, for example provides a means for selective binding of the conjugate. Any IgG binding protein/IgG pair can be used in the present system. Protein A and Protein G are suitable facilitating. Any Protein A or Protein G can be used in the present system.

For example, the following nucleotide sequences can be used for amplifying and constructing Protein A or Protein G fusion proteins:

E04365 (Primer for amplifying IgG binding domain AB of protein A);  
10 E04364 (Primer for amplifying IgG binding domain AB of protein A);  
E01756 (DNA sequence encoding subunit which can bind IgG of protein A like substance); M74187 (Cloning vector pKP497 (cloning, screening, fusion vector) encoding an IgG-binding fusion protein from protein A analogue (ZZ) and beta-Gal' (lacZ) genes). In addition, several Protein A  
15 gene fusion vectors such as pEZ 18 and pRIT2T are commercially available (Amersham Pharmacia Biotech, Inc.).

1) pEZ 18 Protein A gene fusion vector

pEZ 18 Protein A gene fusion vector can be used for rapid expression of secreted fusion proteins and their one-step purification  
20 using IgG Sepharose 6FF. The phagemid pEZ 18 contains the proteins A signal sequence and two synthetic "Z" domains based on the "B" IgG binding domain of Protein A (Löwenadler., et al., *Gene*, 58:87 (1987); and Nilsson., et al., *Prot. Engineering*, 1:107 (1987)). Proteins are expressed as fusions with the "ZZ" peptide and secreted into the aqueous culture medium under the direction of the protein A signal sequence.  
25 They are easily purified using IgG Sepharose 6FF to which the "ZZ" domain binds tightly. Because of its unique folding properties, the 14 kDa "ZZ" peptide has little effect on folding of the fusion partner into a native conformation.

**Expression**

5 Expression is controlled by the *lacUV5* and protein A promoters and is not inducible. Elements of the protein A gene provide the ATG and ribosome-binding sites. Stop codons must be provided by the insert.

**Sequencing**

10 The M13 Universal Sequencing Primer is used for double-stranded and single-stranded sequencing. A protocol for production of single-stranded DNA is provided with the vector.

**Cloning**

Inserts containing a stop codon will yield white colonies when grown on media containing X-gal.

**Host(s)**

15 *E. coli* strains carrying a *lac* deletion but capable of  $\alpha$ -complementation of *lacZ'*.

**Selectable marker(s)**

Plasmid confers resistance to ampicillin.

**Amplification**

Amplification, though not necessarily required can be included.

20 2) pRIT2T Protein A gene fusion vector

The pRIT2T Protein A gene fusion vector (available from Pharmacia) can be used for high-level expression of intracellular fusion proteins. pRIT2T, a derivative of pRIT2 (Nilsson., et al., *EMBO J.*, 4:1075 (1985)), contains the IgG-binding domains of *staphylococcal* 25 protein A which permits rapid affinity purification of fusion proteins on IgG Sepharose 6 FF. Thermo-inducible expression of the fusion protein is achieved in a suitable *E. coli* host strain which carries the temperature-sensitive repressor cI857 (N4830-1) (Zabeau and Stanley, *EMBO J.*, 1:1217 (1982)).

**Induction**

The  $\lambda P_R$  promoter is induced by shifting the growth temperature from 30°C to 42°C for 90 minutes.

**Expression**

5      Genes inserted into the MCS are expressed from the  $\lambda$  right promoter ( $P_R$ ) as fusions with the IgG-binding domains of *staphylococcal* protein A. A portion of the  $\lambda cro$  gene, fused to the IgG-binding domain, supplies the ATG start codon. Since no signal sequence is provided, the protein remains intracellular. Protein A  
10     gene transcription and translation termination signals are provided. Fusion protein can be purified on IgG Sepharose 6FF (17-0969-01). The protein A carrier protein is ~30 kDa.

**Host(s)**

15     *E. coli* N4830-1/N99cl<sup>+</sup>. Supplied with *E. coli* N4830-1 which contains the temperature-sensitive *c/857* repressor.

**Selectable marker(s)**

Plasmid confers resistance to ampicillin.

**3) The IgG Sepharose 6 fast flow system**

The Protein A and Protein G fusion protein can be isolated or  
20     purified by affinity binding with IgG, such as the IgG Sepharose 6 Fast Flow System (Amersham Pharmacia Biotech, Inc.). The IgG Sepharose 6 Fast Flow System includes IgG coupled to the highly cross-linked 6% agarose matrix Sepharose 6 Fast Flow, and is designed for the rapid purification of Protein A and Protein A fusion conjugates. The system  
25     binds at least 2 mg Protein A/ml drained gel with flow possible rates of 300 cm/hr at 1 bar (14.5 psi, 0.1 MPa) in an XK 50/30 column (Lundström et al., *Biotechnology and Bioengineering*, 36:1056 (1990)).

d.  $\beta$ -galactosidase fusion proteins

The pMC1871 fusion vector (commercially available from Pharmacia, see, also Shapira *et al.* *Gene* 25:71 (1983); Casadaban *et al.* *Methods Enzymol.* 100:293 (1983)) for production of enzymatically active  $\beta$ -galactosidase hybrid proteins for gene expression or functional studies. Vector pMC1871 is derived from pBR322 and contains a promoterless lacZ gene, which also lacks a ribosome-binding site and the first eight non-essential N-terminal amino acid codons. Its unique Sma I site allows fusions to the N-terminal part of the  $\beta$ -galactosidase gene.

Insertion of a gene into the *E. coli* lacZ gene results in the production of a hybrid protein, whose presence can be readily detected by following its  $\beta$  galactosidase activity (Miller, J.H., in Experiments in Molecular Gener. (Cold Spring Harbor, N.Y.) (1972); Nielsen *et al.* *Proc. Natl. Acad. Sci. U.S.A.*, 80:5198 (1983)). Hybrid proteins can then be easily purified by affinity chromatography (Germino *et al.* *Proc. Natl. Acad. Sci. U.S.A.*, 81: 4692 (1984)). Multiple cloning sites flanking the lacZ gene permit its excision as a BamH I, Sal I, Pst I or EcoR I gene cassette. If lacZ is excised as an EcoRI cassette, a portion of its 3'-end will be deleted. The resulting  $\beta$ -galactosidase protein ( $\alpha$ -donor) will be functional if the C-terminus of the  $\beta$ -galactosidase protein ( $\alpha$ -acceptor) is available through intercistronic complementation.

Expression

Inserts cloned into the unique Sma I site give fusion proteins with the N-terminal part of  $\beta$ -galactosidase. Insert must contain a promoter, ATG and ribosome-binding site.

Host(s)

*E. coli* strains carrying a lac deletion.

Selectable marker(s)

Plasmid confers resistance to 15  $\mu$ g/ml tetracycline.

GenBank Accession Number L08936.

e. Nucleic acid binding moieties

In another embodiment, the conjugate includes a nucleotide binding protein, peptide or effective fragment thereof as a facilitating agent. The specific binding partner can be nucleotide sequences generally, a set of 5 nucleotide sequences or a particular nucleotide sequence. Any protein-nucleotide interaction pair can be used in the present system. For example, the protein-nucleotide interaction pair can be protein/DNA or protein/RNA pairs, or a combination thereof. Protein-nucleotide interaction pairs can be designed, screened or selected according to the 10 methods known in the art (*See generally, Current Protocols in Molecular Biology* (1998) § 12, John Wiley & Sons, Inc.). Examples of such methods for identifying protein-nucleotide interactions include the gel mobility shift assay, methylation and uracil interference assay, DNase I footprint analysis, λgt11 expression library screening and rapid separation 15 of protein-bound DNA from free DNA using nitrocellulose filters.

1) DNA binding proteins

The conjugate can contain a DNA binding protein and its specific binding partner can be DNA molecules generally, a set of DNA molecules or a particular sequence of nucleotides. Any DNA binding protein can be 20 used in the present system. For example, the DNA binding protein can bind to a single-stranded or double-stranded DNA sequence, or to an A-, B- or Z-form DNA sequence. The DNA binding sequence can also bind to a DNA sequence that is involved in replication, transcription, DNA repair, recombination, transposition or DNA structure maintenance. The DNA 25 binding sequence can further be derived from a DNA binding enzyme such as a DNA polymerase, a DNA-dependent RNA polymerase, a DNAase, a DNA ligase, a DNA topoisomerase, a transposase, a DNA kinase, or a restriction enzyme.

Any DNA binding sequence/DNA sequence pair can be designed, 30 screened or selected according to the methods known in the art including methods described in Section L.2. above.

The following Table 4 illustrates certain properties of several DNA binding sequence/DNA sequence pair systems.

**Table 4. Examples of DNA binding sequence/DNA sequence binding pairs**

	DNA binding sequence	DNA binding sequence motif	DNA sequence	Reference (U.S. Patent No.)
5	NF-AT <sub>p</sub> (SEQ ID NO. 14)	T lymphocyte DNA-binding protein	GCCCCAAAGAGGAA AATTGTTCATAC AG (SEQ ID NO. 15)	5,656,452
10	Max (SEQ ID NO. 16)	helix-loop-helix zipper protein	CACGTG	5,693,487
15	Chicken Lung 140 Kd Protein		Z-DNA	5,726,050
20	EGR1, EGR2, GLI, Wilm's tumor gene, Sp1, Hunchback, Kruppel, ADR1 and BrLA	Zinc finger proteins	GACC, GCAC	5,789,538
25	LIL-Stat protein	Stat family of transcription factors	TTNCNNAGA, TTCCTGAGA	5,821,053
30	Egr (SEQ ID NO. 17)	zinc finger protein	CGCCCCCGC	5,866,325
	S1-3 protein (SEQ ID NO. 18)	zinc finger protein	CATRRWWG	5,905,146

## 2) RNA binding proteins

In another preferred embodiment, the conjugate can contain an RNA binding protein and its specific binding partner can be RNA generally, a set of RNA molecules or a particular sequence of ribonucleotides. Any RNA binding protein can be used in the present system. For example, the RNA binding protein can bind to a single-stranded or double-stranded RNA, or to rRNA, mRNA or tRNA. The RNA binding protein may specifically bind to a RNA that is involved in reverse transcription, transcription, RNA editing, RNA splicing, translation, RNA stabilization, RNA destabilization, or RNA localization. The RNA binding

protein can be derived from or be an RNA binding enzyme such as a RNA-dependent DNA polymerase, a RNA-dependent RNA polymerase, a RNase, a RNA ligase, a RNA maturase, or a ribosome.

Other RNA recognition sequence or binding motifs that can be used

5 in the present system include the zinc-finger motif, the Y-box, the KH motif, AUUUA, histone, RNP motif (U1), arginine-rich motif (ARM or PRE), double-stranded RNA binding motifs (IRE) and RGG box (APP) (U.S. Patent Nos. 5,834,184, 5,859,227 and 5,858,675). The RNP motif is a 90-100 amino acid sequence that is present in one or more copies in

10 proteins that bind pre mRNA, mRNA, pre-ribosomal RNA and snRNA. The consensus sequence and the sequences of several exemplary proteins containing the RNP motif are provided in Burd and Dreyfuss, *Science*, 265:615-621 (1994); Swanson et al., *Trends Biochem. Sci.*, 13:86 (1988); Bandziulis et al., *Genes Dev.*, 3:431 (1989); and Kenan et al., *Trends Biochem. Sci.*, 16:214 (1991). The RNP consensus motif contains two short consensus sequences RNP-1 and RNP-2. Some RNP proteins bind specific RNA sequences with high affinities (dissociation constant in the range of  $10^{-8}$ - $10^{-11}$  M). Such proteins often function in RNA processing reactions. Other RNP proteins have less stringent

15 sequence requirements and bind less strongly (dissociation constant about  $10^{-6}$ - $10^{-7}$  M) (Burd & Dreyfuss, *EMBO J.*, 13:1197 (1994)).

A second characteristic RNA binding motif found in viral, phage and ribosomal proteins is an arginine-rich motif (ARM) of about 10-20 amino acids. RNA binding proteins having this motif include the HIV Tat and Rev proteins. Rev binds with high affinity disassociation constant ( $10^{-9}$  M) to an RNA sequence termed RRE, which is found in all HIV mRNAs (Zapp et al., *Nature*, 342:714 (1989); and Dayton et al., *Science*, 246:1625 (1989)). Tat binds to an RNA sequence termed TAR with a dissociation constant of  $5 \times 10^{-9}$  M (Churcher et al., *J. Mol. Biol.*, 230:90 (1993)). For Tat and Rev proteins, a fragment containing the arginine-rich motif binds as strongly as the intact protein. In other RNA

binding proteins with ARM motifs, residues outside the ARM also contribute to binding.

The double-stranded RNA-binding domain (dsRBD) exclusively binds double-stranded RNA or RNA-DNA. A dsRBD motif includes a

5 region of approximately 70 amino acids which includes basic residues and contains a conserved core sequence with a predicted  $\alpha$ -helical structure. The dsRBD motif is found in at least 20 known or putative RNA-binding proteins from different organisms. There are two types of dsRBDs; Type A, which is homologous along its entire length with the defined

10 consensus sequence, and Type B, which is more highly conserved at its C terminus than its N terminus. These domains have been functionally delineated in specific proteins by deletion analysis and RNA binding assays (St Johnston, et al., *Proc. Natl. Acad. Sci.*, 89:10979-10983 (1992)).

15 Any RNA binding sequence/RNA sequence pair can be designed, screened or selected according to the methods known in the art including the methods described in Section L.2. above and the methods, such as those described in U.S. Patent Nos. 5,834,184 and 5,859,227, and in SenGupta et al., A three-hybrid system to detect RNA-protein interactions

20 in vivo, *Proc. Nat. Acad. Sci. U.S.A.*, 93:8496-8501 (1996)).

For example, U.S. Patent No. 5,834,184 describes a method of screening a plurality of polypeptides for RNA binding activity. The method includes the steps of: (1) culturing a library of prokaryotic cells that constitute a library, and (2) detecting expression of the reporter gene

25 in a cell from the library, the expression indicating that the cell comprises a polypeptide having RNA binding activity. The cells contain at least one vector that contains a first DNA segment that encodes a fusion protein of a prokaryotic anti-terminator protein having anti-terminator activity linked in-frame to the test polypeptide, which varies among the cells in the

30 library, that is operably linked to a second DNA segment. The second DNA segment contains a promoter, an RNA recognition sequence foreign

to the anti-terminator protein, a transcription termination site and a reporter gene. The termination site blocks transcription of the reporter gene in the absence of a protein with anti-termination activity and affinity for the RNA recognition sequence. If the test polypeptide has specific 5 affinity for the recognition sequence, it binds via the polypeptide to the RNA recognition sequence of a transcript from the second DNA segment thereby inducing transcription of the second DNA segment to proceed through the termination site to the reporter gene resulting in expression of the reporter gene.

- 10 U.S. Patent No. 5,859,227 describes methods for identifying possible binding sites for RNA binding proteins in nucleic acid molecules, and confirming the identity of such prospective binding sites by detection of interaction between the prospective binding site and RNA binding proteins. These methods involve identification of possible binding sites 15 for RNA binding proteins, by either searching databases for untranslated regions of gene sequences or cloning untranslated sequences using a single specific primer and an universal primer, followed by confirmation that the untranslated regions in fact interact with RNA binding proteins using the RNA/RBP detection assay. Genomic nucleic acid can further be 20 screened for putative binding site motifs in the nucleic acid sequences. Information about binding sites that are confirmed in the assay then can be used to redefine or redirect the nucleic acid sequence search criteria, for example, by establishing or refining a consensus sequence for a given binding site motif.
- 25 SenGupta et al., *Proc. Nat. Acad. Sci. U.S.A.*, 93:8496-8501 (1996) describes a yeast genetic method to detect and analyze RNA-protein interactions in which the binding of a bifunctional RNA to each of two hybrid proteins activates transcription of a reporter gene *in vivo* (see also Wang et al., *Genes & Dev.*, 10:3028-3040 (1996)).
- 30 SenGupta et al. demonstrate that this three-hybrid system enables the rapid, phenotypic detection of specific RNA-protein interactions. As

examples, SenGupta et al. use the binding of the iron regulatory protein 1 (IRP1) to the iron response element (IRE), and of HIV trans-activator protein (Tat) to the HIV trans-activation response element (TAR) RNA sequence. The three-hybrid assay relies only on the physical properties of 5 the RNA and protein, and not on their natural biological activities; as a result, it may have broad application in the identification of RNA-binding proteins and RNAs, as well as in the detailed analysis of their interactions.

The following Table 5 illustrates certain properties of several RNA 10 binding sequence/RNA sequence pair systems.

**Table 5. Examples of RNA binding sequence/RNA sequence pairs**

RNA binding sequence	RNA binding sequence motif	RNA sequence	Reference (U.S. Patent No.)
BINDR	double-stranded RNA-binding	double-stranded RNA poly(rI) and poly (rC)	5,858,675
15 Protein extract from SH-SY5Y cells	5' untranslated region (UTR)	UTR of Glut1 (SEQ ID NO. 19); 5' UTR of (HMG,CoA Red) (SEQ ID NO. 20); 5' UTR of human C4b-binding $\alpha$ chain (SEQ ID NO. 21); 5' UTR of human CD45 (SEQ ID NO. 22)	5,859,227

### 3) Preparation of nucleic acid binding proteins

Extracts prepared from the isolated nuclei of cultured cells are 20 functional in accurate *in vitro* transcription and mRNA processing (See generally, *Current Protocols in Molecular Biology* (1998) § 12.1., John Wiley & Sons, Inc.). Thus, such extracts can be used directly for functional studies and as the starting material for purification of the proteins involved in these processes. To prepare nuclear extracts, tissue 25 culture cells are collected, washed, and suspended in hypotonic buffer. The swollen cells are homogenized and nuclei are pelleted. The cytoplasmic fraction is removed and saved, and nuclei are resuspended in

a low-salt buffer. Gentle dropwise addition of a high-salt buffer then releases soluble proteins from the nuclei (without lysing the nuclei). Following extraction, the nuclei are removed by centrifugation, the nuclear extract supernatant is dialyzed into a moderate salt solution, and 5 any precipitated protein is removed by centrifugation.

The nuclear and cytoplasmic extraction procedure (see, e.g., Dignam et al., 1983, *Nucl. Acids. Res.* 11:1475-1489 (Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei); Dignam, et al., 1983, *Methods Enzymol.* 101:582-598 (Eukaryotic gene transcription with purified components); Krainer, et al., 1984, *Cell* 36:993-1005 (Normal and mutant human  $\beta$ -globin pre-mRNAs are faithfully and efficiently spliced in vitro); Lue, et al., 1987, *Proc. Natl. Acad. Sci. U.S.A.* 84:8839-8843 (Accurate initiation at RNA polymerase II promoters in extracts from *Saccharomyces cerevisiae*); 10 Manley, et al., 1980, *Proc. Natl. Acad. Sci. U.S.A.* 77:3855-3859 (DNA-dependent transcription of adenovirus genes in a soluble whole-cell extract); Weil, et al., 1979, *J. Biol. Chem.* 254:6163-6173 (Faithful transcription of eukaryotic genes by RNA polymerase III in systems reconstituted with purified DNA templates); and Weil, et al., 1979, *Cell* 15:469-484 (Selective and accurate initiation of transcription at the Ad2 major late promotor in a soluble system dependent on purified RNA polymerase II and DNA)) and the identified protein-DNA interaction pairs can be used in the present system.

4) Assays for identifying nucleic acid binding proteins  
25

a) Mobility shift DNA-binding assay

The DNA-binding assay using nondenaturing polyacrylamide gel electrophoresis (PAGE) provides a simple, rapid, and extremely sensitive method for detecting sequence-specific DNA-binding proteins (See 30 generally, *Current Protocols in Molecular Biology* (1998) § 12.2., John Wiley & Sons, Inc.). Proteins that bind specifically to an end-labeled DNA

fragment retard the mobility of the fragment during electrophoresis, resulting in discrete bands corresponding to the individual protein-DNA complexes. The assay can be used to test binding of purified proteins or of uncharacterized factors found in crude extracts. This assay also

5 permits quantitative determination of the affinity, abundance, association rate constants, dissociation rate constants, and binding specificity of DNA-binding proteins.

b) Basic mobility shift assay procedure

The basic mobility shift assay procedure includes 4 steps: (1)

10 preparation of a radioactively labeled DNA probe containing a particular protein binding site; (2) preparation of a nondenaturing gel; (3) a binding reaction in which a protein mixture is bound to the DNA probe; and (4) electrophoresis of protein-DNA complexes through the gel, which is then dried and autoradiographed. The mobility of the DNA-bound protein is

15 retarded while that of the non-bound protein is not retarded.

c) Competition mobility shift assay

One important aspect of the mobility shift DNA-binding assay is the ease of assessing the sequence specificity of protein-DNA interactions using a competition binding assay. This is necessary because most

20 protein preparations will contain specific and nonspecific DNA binding proteins. For a specific competitor, the same DNA fragment (unlabeled) as the probe can be used. The nonspecific competitor can be essentially any fragment with an unrelated sequence, but it is useful to roughly match the probe and specific competitor for size and configuration of the

25 ends. For example, some proteins bind blunt DNA ends nonspecifically. These would not be competed by circular plasmid or a fragment with overhangs, leading to the false conclusion that the protein-DNA complex represented specific binding. Perhaps the best control competitor is a

30 DNA fragment that is identical to the probe fragment except for a mutation(s) in the binding site that is known to disrupt function (and presumably binding).

d) **Antibody supershift assay**

Another useful variation of the mobility shift DNA-binding assay is to use antibodies to identify proteins present in the protein-DNA complex. Addition of a specific antibody to a binding reaction can have one of 5 several effects. If the protein recognized by the antibody is not involved in complex formation, addition of the antibody should have no effect. If the protein that forms the complex is recognized by the antibody, the antibody can either block complex formation, or it can form an antibody-protein-DNA ternary complex and thereby specifically result in a further 10 reduction in the mobility of the protein-DNA complex (supershift).

Results may be different depending upon whether the antibody is added before or after the protein binds DNA (particularly if there are epitopes on the DNA-binding surface of the protein).

The mobility shift DNA-binding assay has been successfully 15 employed (see, e.g., Carthew, et al., 1985, *Cell* 43:439-448 (An RNA polymerase II transcription factor binds to an upstream element in the adenovirus major late promoter); Chodosh, et al., 1986, *Mol. Cell. Biol.* 6:4723-4733 (A single polypeptide possesses the binding and activities of the adenovirus major late transcription factor); Fried, et al., 1981, 20 *Nucl. Acids. Res.*, 9:6505-6525 (Equilibria and kinetics of *lac* repressor-operator interactions by polyacrylamide gel electrophoresis); Fried, et al., 1984, *J. Mol. Biol.* 172:241-262 (Kinetics and mechanism in the reaction of gene regulatory proteins with DNA); Fried, et al., 1984, *J. Mol. Biol.* 172:263-282 (Equilibrium studies of the cyclic AMP receptor protein-DNA 25 interaction); Garner, et al., 1981, *Nucl. Acids Res.* 9:3047-3060 (A gel electrophoresis method for quantifying the binding of proteins to specific DNA regions: Application to components of the *Escherichia coli* lactose operon regulatory system); Hendrickson, et al., 1984, *J. Mol. Biol.* 174:611-628 (Regulation of the *Escherichia coli* L-arabinose operon 30 studied by gel electrophoresis DNA binding assay); Kristie, et al., 1986, *Proc. Natl. Acad. Sci. U.S.A.* 83:3218-3222 (The major regulatory

protein of herpes simplex virus type 1, is stably and specifically associated with promoter-regulatory domains of a genes and/or selected viral genes); Lieberman, et al., 1994, *Genes & Dev.* 8:995-1006 (A mechanism for TAFs in transcriptional activation: Activation domain enhancement of TFIID-TFIIB-promoter DNA complex formation); Riggs, et al., 1970, *J. Mol. Biol.* 48:67-83 (Lac repressor-operator interactions: I. Equilibrium studies); Singh, et al., 1986, *Nature* 319:154-158 (A nuclear factor that binds to a conserved sequence motif in transcriptional control elements of immunoglobulin genes); Staudt, et al., 1986, *Nature* 323:640-643 (A lymphoid-specific protein binding to the octamer motif of immunoglobulin genes); Strauss, et al., 1984, *Cell* 37:889-901 (A protein binds to a satellite DNA repeat at three specific sites that would be brought into mutual proximity by DNA folding in the nucleosome); and Zinkel, et al., 1987, *Nature* 328:178-181 (DNA bend direction by phase-sensitive detection)) and the identified protein-DNA interaction pairs can be used in the present system.

e) **Methylation and uracil interference assay**

Interference assays identify specific residues in the DNA binding site that, when modified, interfere with binding of the protein (See generally, *Current Protocols in Molecular Biology* (1998) § 12.3., John Wiley & Sons, Inc.). These protocols use end-labeled DNA probes that are modified at an average of one site per molecule of probe. These probes are incubated with the protein of interests, and protein-DNA complexes are separated from free probe by the mobility shift assay. A DNA probe that is modified at a position that interferes with binding will not be retarded in this assay; thus, the specific protein-DNA complex is depleted for DNA that contains modifications on bases important for binding. After gel purification the bound and unbound DNA are specifically cleaved at the modified residues and the resulting products analyzed by electrophoresis on polyacrylamide sequencing gels and autoradiography. These procedures provide complementary information

about the nucleotides involved in protein-DNA interactions.

**1) Methylation interference assays**

In methylation interference, probes are generated by methylating guanines (at the N-7 position) and adenines (at the N-3 position) with DMS; these methylated bases are cleaved specifically by piperidine. Methylation interference identifies guanines and adenines in the DNA binding site that, when methylated, interfere with binding of the protein. The protocol uses a single end-labeled DNA probe that is methylated at an average of one site per molecule of probe. The labeled probe is a substrate for a protein-binding reaction. DNA-protein complexes are separated from the free probe by the mobility shift DNA-binding assay. A DNA probe that is methylated at a position that interferes with binding will not be retarded in this assay. Therefore, the specific DNA-protein complex is depleted for DNA that contains methyl groups on purines important for binding. After gel purification, DNA is cleaved with piperidine. Finally, these fragments are electrophoresed on polyacrylamide sequencing gels and autoradiographed. Guanines and adenines that interfere with binding are revealed by their absence in the retarded complex relative to a lane containing piperidine-cleaved free probe. This procedure offers a rapid and highly analytical means of characterizing DNA-protein interactions.

**2) Uracil interference assay**

In uracil interference, probes are generated by PCR amplification in the presence of a mixture of TTP and dUTP, thereby producing products in which thymine residues are replaced by deoxyuracil residues (which contains hydrogen in place of the thymine 5-methyl group). Uracil bases are specifically cleaved by uracil-N-glycosylase to generate apyrimidinic sites that are susceptible to piperidine. Uracil interference identifies thymines in a DNA binding site that, when modified, interfere with binding of the protein. Probes generated by PCR amplification in the presence of TTP and dUTP incorporate deoxyuracil in place of thymine

residues. PCR products are incubated with the binding protein and resulting complexes are separated from unbound DNA. The DNA recovered from the protein-DNA complex is treated with uracil-N-glycosylase and piperidine, and the products are then electrophoresed on 5 a denaturing polyacrylamide gel.

The methylation and uracil interference assays have been successfully used (see, e.g., Baldwin, et al., 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:723-727 (Two transcription factors, H2TF1 and NF- $\kappa$ B, interact with a single regulatory sequence in the class I MHC promoter); 10 Brunelle, et al., 1987, *Proc. Natl. Acad. Sci. U.S.A.* 84:6673-6676 (Missing contact probing of DNA-protein interactions); Goeddel, et al., 1978, *Proc. Natl. Acad. Sci. U.S.A.* 75:3579-3582 (How lac repressor recognizes lac operator); Ivarie, et al., 1987, *Nucl. Acids Res.* 15:9975-9983 (Thymine methyls and DNA-protein interactions); Maxam, et al., 15 1980, *Methods Enzymol.* 65:499-560 (Sequencing end-labeled DNA with base-specific chemical cleavages); Pu, et al., 1992, *Nucl. Acids Res.* 20:771-775 (Uracil interference, a rapid and general method for defining protein-DNA interactions involving the 5-methyl group of thymines: The GCN4-DNA complex); Siebenlist, et al., 1980, *Proc. Natl. Acad. Sci. U.S.A.* 77:122-126 (Contacts between *E. coli* RNA polymerase and an early promoter of phase T7); and Hendrickson, et al., 1985, *Proc. Natl. Acad. Sci. U.S.A.* 82:3129-3133 (A dimer of AraC protein contacts three adjacent major groove regions at the Ara I DNA site)) and the identified protein-DNA interaction pairs can be used in the present system.

25 3) DNase I footprint analysis

Deoxyribonuclease I (DNase I) protection mapping, or footprinting, is a valuable technique for locating the specific binding sites of proteins on DNA (See generally, *Current Protocols in Molecular Biology* (1998) § 12.4., John Wiley & Sons, Inc.). The basis of this assay is that bound 30 protein protects that phosphodiester backbone of DNA from DNase I catalyzed hydrolysis. Binding sites are visualized by autoradiography of

the DNA fragments that result from hydrolysis, following separation by electrophoresis on denaturing DNA sequencing gels. Footprinting has been developed further as a quantitative technique to determine separate binding curves for each individual protein-binding site on the DNA. For 5 each binding site, the total energy of binding is determined directly from that site's binding curve. For sites that interact cooperatively, simultaneous numerical analysis of all the binding curves can be used to resolve the intrinsic binding and cooperative components of these energies.

10 DNase I footprint analysis has been successfully employed (see, e.g., Ackers, et al., 1982, *Proc. Natl. Acad. Sci. U.S.A.* 79:1129-1133 (Quantitative model for gene regulation by lambda phage repressor); Ackers, et al., 1983, *J. Mol. Biol.* 170:223-242 (Free energy coupling within macromolecules: The chemical work of ligand binding at the

15 individual sites in cooperative systems); Brenowitz, et al., 1986, *Proc. Natl. Acad. Sci. U.S.A.* 83:8462-8466 (Footprint titrations yield valid thermodynamic isotherms.); Brenowitz, et al., 1986, *Meth. Enzymol.* 130:132-181 (Quantitative DNase I footprint titration: A method for studying protein-DNA interactions); Dabrowiak, et al., 1989, *In Chemistry*

20 and Physics of DNA-Ligand Interactions (N.R. Kallenback, ed.) Adenine Press. (Quantitative footprinting analysis of drug-DNA interactions); Galas, et al., 1978, *Nucl. Acids Res.* 5:3157-3170 (DNase footprinting: A simple method for the detection of protein-DNA binding specificity); Hertzberg, et al., 1982, *J. Am. Chem. Soc.* 104:313-315 (Cleavage of

25 double helical DNA by (methidiumpropyl-EDTA) iron (III)); Johnson, et al., 1979, *Proc. Natl. Acad. Sci. U.S.A.* 76:5061-5065 (Interactions between DNA-bound repressors govern regulation by the lambda phage repressor); Johnson, et al., 1985, *Meth. Enzymol.* 117:301-342 (Nonlinear least-squares analysis); Senear, et al., 1986, *Biochemistry* 25:7344-7354

30 (Energetics of cooperative protein-DNA interactions: Comparison between quantitative DNase I footprint titration and filter binding); and Tullius, et

al., 1987, *Meth. Enzymol.* **155**:537-558 (Hydroxyl radical footprinting: A high resolution method for mapping protein-DNA contacts), and the identified protein-DNA interaction pairs can be used in the present system.

5

**4) Screening a  $\lambda$ gt11 expression library with recognition-site DNA**

A clone encoding a sequence-specific protein can be detected in a  $\lambda$ gt11 library because its recombinant protein binds specifically to a radiolabeled recognition-site DNA (See generally, *Current Protocols in*

10 *Molecular Biology* (1998) § 12.7., John Wiley & Sons, Inc.).

Bacteriophage from a cDNA library constructed in the vector  $\lambda$ gt11 are plated under lytic growth conditions. After plaques appear, expression of the  $\beta$ -galactosidase fusion proteins encoded by the recombinant phage is induced by placing nitrocellulose filters impregnated with IPTG onto the

15 plate. Phage growth is continued and is accompanied by the immobilization of proteins, from lysed cells, onto the nitrocellulose filters.

The filters are lifted after this incubation, blocked with protein, then reacted with a radiolabeled recognition-site DNA (containing one or more binding sites for the relevant sequence-specific protein) in the presence of

20 an excess of nonspecific competitor DNA. After the binding reaction, the filters are washed to remove nonspecifically bound probe and processed for autoradiography. Potentially positive clones detected in the primary screen are rescreened after a round of plaque purification. Recombinants which screen positively after enrichment and whose detection specifically

25 requires the recognition-site probe (not detected with control probes lacking the recognition site for the relevant protein) are then isolated by further rounds of plaque purification.

The  $\lambda$ gt11 expression screening methods have been successfully used (see, e.g., Androphy, et al., 1987, *Nature (Lond.)* **325**:70-73

30 (Bovine papillomavirus E2 trans-activating gene product binds to specific sites in papillomavirus DNA); Arndt, et al., 1986, *Proc. Natl. Acad. Sci.*

U.S.A. 83:8516-8520 (GCN4 protein, a positive transcription factor in yeast, binds general control promoters at 5'TGACTC3' sequences); Chodosh, et al., 1988, *Cell* 53:25-35 (A yeast and a human CCAAT-binding protein have heterologous subunits that are functionally interchangeable); Desplan, et al., 1985, *Nature (Lond.)* 318:630-635 (The *Drosophila* developmental gene, engrailed, encodes a sequence-specific DNA binding activity); Hoeffler, et al., 1988, *Science* 242:1430-1433 (Cyclic AMP-responsive DNA-binding protein: Structure based on a cloned placental cDNA); Hsiou-Chi, et al., 1988, *Science* 242:69-71

5 10 15 20 25 30

(Distinct cloned class II MHC DNA binding proteins recognize the X box transcription element); Ingraham, et al., 1988, *Cell* 55:519-529 (A tissue-specific transcription factor containing a homeo domain specifies a pituitary phenotype); Kadonaga, et al., 1987, *Cell* 51:1079-1090 (Isolation of cDNA encoding transcription factor Sp1 an functional analysis of the DNA binding domain); Keegan, et al., 1986, *Science* 231:699-704 (Separation of DNA binding from the transcription-activating function of a eukaryotic regulatory protein); Miyamoto, et al., 1988, *Cell* 54:903-913 (Regulated expression of a gene encoding a nucleic factor, IRF-1, that specifically binds to IFN- $\beta$  gene regulatory elements); Murre, et al., 1989, *Cell* 56:777-783 (A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD and myc proteins); Müller, et al., 1988, *Nature (Lond.)* 336:544-551 (A cloned octamer transcription factor stimulates transcription from lymphoid specific promoters in non-B cells); Rawlins, et al., 1985, *Cell* 42:859-868 (Sequence-specific DNA binding of the Epstein-Barr viral nuclear antigen (EBNA-1) to clustered sites in the plasmid maintenance region); Reith, et al., 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:4200-4204 (Cloning of the major histocompatibility complex class II promoter affected in a hereditary defect in class II gene regulation); Singh, et al., 1988, *Cell* 52:415-423 (Molecular cloning of an enhancer binding protein: Isolation by screening of an expression library with a recognition

site); Staudt, et al., 1988, *Science* 241:577-580 (Molecular cloning of a lymphoid-specific cDNA encoding a protein that binds to the regulatory octamer DNA motif); Sturm, et al., 1988, *Genes & Dev.* 2:1582-1599 (The ubiquitous octamer protein Oct-1 contains a Pou domain with a homeo subdomain); Vinson, et al., 1988, *Genes & Dev.* 2:801-806 (*In situ* detection of sequence-specific DNA binding activity specified by a recombinant bacteriophage); Weinberger, et al., 1985, *Science* 228:740-742 (Identification of human glucocorticoid receptor complementary DNA clones by epitope selection); and Young, et al., 1983, *Science* 222:778-782 (Yeast RNA polymerase II genes: Isolation with antibody probes)) and the identified protein-DNA interaction pairs can be used in the present system.

5) **Rapid separation of protein-bound DNA from free DNA**

15 This method relies on the ability of nitrocellulose to bind proteins but not double-stranded DNA (See generally, *Current Protocols in Molecular Biology* (1998) § 12.8., John Wiley & Sons, Inc.). Use of radioactively labeled double-stranded DNA fragments allows quantitation of DNA bound to the protein at various times and under various 20 conditions, permitting kinetic and equilibrium studies of DNA-binding interactions. Purified protein is mixed with double-stranded DNA in an appropriate buffer to allow interaction. After incubation, the mixture is suction filtered through nitrocellulose, allowing unbound DNA to pass through the filter while the protein (and any DNA interacting with it) is 25 retained.

Nitrocellulose filter methods have been successfully used (see, e.g., Barkley, et al., 1975, *Biochemistry* 14:1700-1712 (Interaction of effecting ligands with *lac* repressor and repressor-operator complex); Fried, et al., 1981, *Nucl. Acids Res.* 9:6505-6525 (Equilibria and kinetics 30 of *lac* repressor-operator interactions by polyacrylamide gel electrophoresis); Hinkle, et al., 1972, *J. Mol. Biol.* 70:157-185 (Studies

of the binding of *Escherichia coli* RNA polymerase to DNA I. The role of sigma subunit in site selection); Hinkle, et al., 1972, *J. Mol. Biol.* 70:187-195 (Studies of the binding of *Escherichia coli* RNA polymerase to DNA II. The kinetics of the binding reaction); Hinkle, et al., 1972, *J. Mol. Biol.*

5   70:197-207 (Studies of the binding of *Escherichia coli* RNA polymerase to DNA III. Tight binding of RNA polymerase holoenzyme to single-strand breaks in T7 DNA); Jones, et al., 1966, *J. Mol. Biol.* 22:199-209 (Studies on the binding of RNA polymerase to polynucleotides); Lin, et al., 1972, *J. Mol. Biol.* 72:671-690 (*Lac* repressor binding to non-

10 operator DNA: Detailed studies and a comparison of equilibrium and rate competition methods); Lin, et al., 1975, *Cell* 4:107-111 (The general affinity of *lac* repressor for *E. coli* DNA: Implications for gene regulation in prokaryotes and eucaryotes); Nirenberg, et al., 1964, *Science* 145:1399-1407 (RNA codewords and protein synthesis: The effect of trinucleotides

15 upon the binding of sRNA to ribosomes); Ptashne, et al., 1987, *A Genetic Switch: Gene Control and Phage λ* pp. 80-83 and 109-118. Cell Press, Cambridge, MA and Blackwell Scientific, Boston, MA; Riggs, et al., 1970, *J. Mol. Biol.* 48:67-83 (*Lac* repressor-operator interactions: I. Equilibrium studies); Strauss, et al., 1980, *Biochemistry* 19:3496-3504 (Binding of

20 *Escherichia coli* ribonucleic acid polymerase holoenzyme to a bacteriophage T7 promoter-containing fragment: Selectivity exists over a wide range of solution conditions); Strauss, et al., 1980, *Biochemistry* 19:3504-3515 (Binding of *Escherichia coli* ribonucleic acid polymerase holoenzyme to a bacteriophage T7 promoter-containing fragment:

25 Evaluation of promoter binding constants as a function of solution conditions); and Strauss, et al., 1981, *Gene* 13:75-87 (Variables affecting the selectivity and efficiency of retention of DNA fragments by *E. coli* RNA polymerase in the nitrocellulose-filter binding assay)) and the identified protein-DNA interaction pairs can be used in the present

30 system.

f. **Lipid binding moieties**

The conjugate can also contain a lipid binding protein, peptide or effective fragment thereof. Its specific binding partner can be lipids generally, a set of lipids or a particular lipid. Any lipid binding moiety, particularly proteins, peptides or effective fragments thereof can be used in the present system. For example, the lipid binding protein can bind to a triacylglycerol, a wax, a phosphoglyceride, a sphingolipid, a sterol and a sterol fatty acid ester. More preferably, the lipid binding sequence comprises a C2 motif or an amphipathic  $\alpha$ -helix motif.

Any lipid binding sequence/lipid pair can be designed, screened or selected according to the methods known in the art (see, e.g., Kane et al., *Anal. Biochem.*, 233(2):197-204 (1996); Arnold et al., *Biochim. Biophys. Acta*, 1233(2):198-204 (1995); Miller and Cistola, *Mol. Cell. Biochem.*, 123(1-2):29-37 (1993); and Teegarden et al., *Anal. Biochem.*, 199(2):293-9 (1991).

For example, Kane et al., *Anal. Biochem.*, 233(2):197-204 (1996) describes that the fluorescent probe 1-anilinonaphthalene 8-sulfonic acid (1,8-ANS) has been used to characterize a general assay for members of the intracellular lipid-binding protein (iLBP) multigene family. The adipocyte lipid-binding protein (ALBP), the keratinocyte lipid-binding protein (KLBP), the cellular retinol-binding protein (CRBP), and the cellular retinoic acid-binding protein I (CRABPI) have been characterized as to their ligand binding activities using 1,8-ANS. ALBP and KLBP exhibited the highest affinity probe binding with apparent dissociation constants (Kd) of 410 and 530 nM, respectively, while CRBP and CRABPI bound 1,8-ANS with apparent dissociation constants of 7.7 and 25 microM, respectively. In order to quantitate the fatty acid and retinoid binding specificity and affinity of ALBP, KLBP, and CRBP, a competition assay was developed to monitor the ability of various lipid molecules to displace bound 1,8-ANS from the binding cavity. Oleic acid and arachidonic acid displaced bound 1,8-ANS from ALBP, with apparent inhibitor constants

(Ki) of 134 nM, while all-trans-retinoic acid exhibited a seven-fold lower Ki (870 nM). The short chain fatty acid octanoic acid and all-trans-retinol did not displace the fluorophore from ALBP to any measurable extent. In comparison, the displacement assay revealed that KLBP bound oleic acid and arachidonic acid with high affinity (Ki = 420 and 400 nM, respectively) but bound all-trans-retinoic acid with a markedly reduced affinity (Ki = 3.6 microM). Like that for ALBP, neither octanoic acid nor all-trans-retinol were bound by KLBP. Displacement of 1,8-ANS from CRBP by all-trans-retinal and all-trans-retinoic acid yielded Ki values of 1.7 and 5.3 microM, respectively. These results indicate the utility of the assay for characterizing the ligand binding characteristics of members of the iLBP family and suggests that this technique may be used to characterize the ligand binding properties of other hydrophobic ligand binding proteins.

15        Arnold et al., *Biochim. Biophys. Acta*, 1233(2):198-204 (1995) describes an assay for analyzing the specific binding of proteins to lipid ligands contained within vesicles or micelles. This assay, referred to as the electrophoretic migration shift assay, was developed using a model system composed of cholera toxin and of its physiological receptor,

20        monosialoganglioside GM1. Using polyacrylamide gel electrophoresis in non-denaturing conditions, the migration of toxin components known to interact with GM1 was retarded when GM1 was present in either lipid vesicles or micelles. This effect was specific, as the migration of proteins not interacting with GM1 was not modified. The localization of retarded

25        proteins and of lipids on gels was further determined by autoradiography. The stoichiometry of binding between cholera toxin and GM1 was determined, giving a value of five GM1 per one pentameric assembly of cholera toxin B-subunits, in agreement with previous studies. The general applicability of this assay was further established using streptavidin and

30        annexin V together with specific lipid ligands. This assay is fast, simple, quantitative, and requires only microgram quantities of protein.

Miller and Cistola, *Mol. Cell. Biochem.*, 123(1-2):29-37 (1993) teaches that titration calorimetry can be used as a method for obtaining binding constants and thermodynamic parameters for the cytosolic fatty acid- and lipid-binding proteins. A feature of this method is its ability to

5 accurately determine binding constants in a non-perturbing manner. This is achieved because the assay does not require separation of bound and free ligand to obtain binding parameters. Also, the structure of the lipid-protein complex was not perturbed, since native ligands were used rather than non-native analogues. As illustrated for liver fatty

10 acid-binding protein, the method distinguished affinity classes whose dissociation constants differed by an order of magnitude or less. It also distinguished endothermic from exothermic binding reactions, as illustrated for the binding of two closely related bile salts to ileal lipid-binding protein. The main limitations of the method are its relatively

15 low sensitivity and the difficulty working with highly insoluble ligands, such as cholesterol or saturated long-chain fatty acids. The signal-to-noise ratio was improved by manipulating the buffer conditions, as illustrated for oleate binding to rat intestinal fatty acid binding protein.

Teegarden et al., *Anal. Biochem.*, 199(2):293-9 (1991) describes

20 an assay for measurement of the affinity of serum vitamin D binding protein for 25-hydroxyvitamin D<sub>3</sub>, 1,25-dihydroxyvitamin D<sub>3</sub>, and vitamin D<sub>3</sub>, using uniform diameter (6.4 microns) polystyrene beads coated with phosphatidylcholine and vitamin D metabolites as the vitamin D donor. The lipid metabolite coated beads have a solid core, and thus all of the

25 vitamin D metabolites are on the bead surface from which transfer to protein occurs. After incubating these beads in neutral buffer for 3 h, essentially no <sup>3</sup>H-labeled vitamin D metabolites desorb from this surface. Phosphatidylcholine/vitamin D metabolite-coated beads (1 microM vitamin D metabolite) were incubated with varying concentrations of serum

30 vitamin D binding protein under conditions in which the bead surfaces were saturated with protein, but most of the protein was free in solution.

After incubation, beads were rapidly centrifuged without disturbing the equilibrium of binding and vitamin D metabolite bound to sDBP in solution was assayed in the supernatant. All three vitamin D metabolites became bound to serum vitamin D binding protein, and after 10 min of incubation

5 the transfer of the metabolites to serum vitamin D binding protein was time independent. The transfer followed a Langmuir isotherm, and the Kd for each metabolite binding to serum vitamin D binding protein was derived by nonlinear least-squares fit analysis. From this analysis the following values for the Kd were obtained:  $5.59 \times 10^{-6}$  M,

10 25-hydroxyvitamin D;  $9.45 \times 10^{-6}$  M, 1,25-dihydroxyvitamin D; and  $9.17 \times 10^{-5}$  M, vitamin D. The method disclosed herein avoids problems encountered in previous assays and allows the precise and convenient determination of binding affinities of vitamin D metabolites and serum vitamin D binding protein.

15 In addition, known protein/lipid binding pairs can be used in the methods and with the products provided herein (see, e.g., Hinderliter et al., *Biochim. Biophys. Acta*, 1448(2):227-35 (1998) (C2 motif binds phospholipid in a manner that is modulated by Ca<sup>2+</sup> and confers membrane-binding ability on a wide variety of proteins, primarily proteins involved in signal transduction and membrane trafficking events);

20 Campagna et al., *J. Dairy Sci.*, 81(12):3139-48 (1998) (an amphipathic helical lipid-binding motif of a glycosylated phosphoprotein, component PP3 in bovine milk); Chae et al., *J. Biol. Chem.*, 273(40):25659-63 (1998) (The C2A domain of synaptotagmin I, which binds Ca<sup>2+</sup> and anionic phospholipids); Johnson et al., *Biochemistry*, 37(26):9509-19 (1998) (the membrane binding domain of phosphocholine cytidylyltransferase (CT) includes a continuous amphipathic alpha-helix between residues approximately 240-295 anionic lipids); Kiyosue et al., *Plant Mol. Biol.*, 35(6):969-72 (1997) (Ca<sup>2+</sup>-dependent lipid-binding

25 domains of cytosolic phospholipase A2, protein kinase C, Rabphilin-3A, and Synaptotagmin 1 of animals); Welters et al., *Proc. Natl. Acad. Sci.*

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USA, 91(24):11398-402 (1994) (calcium-dependent lipid-binding domain is near the N terminus of phosphatidylinositol (PI) 3-kinase cloned from *Arabidopsis thaliana*); and Filoteo et al., *J. Biol. Chem.*, 267(17):11800-5 (1992) (Peptide G25:

5 LysLysAlaValLysValProLysLysGluLysSerValLeuGlnGlyLysLeuThrArgLeuAlaValGlnIle  
(SEQ ID No. 23) representing the putative lipid-binding region (G region) of the erythrocyte Ca<sup>2+</sup> pump interacted with acidic lipids, as shown by the increase in size of phosphatidylserine liposomes in its presence)).

g. Polysaccharide binding moieties

10 The conjugate can include a polysaccharide binding protein, peptide or effective fragment thereof. Its specific binding partner can be polysaccharides generally, a set of polysaccharides or a particular polysaccharide. Any polysaccharide binding moiety, such as a protein, can be used in the present system and include but are not limited to a 15 polysaccharide binding sequence that binds to starch, glycogen, cellulose or hyaluronic acid.

Any polysaccharide binding protein/polysaccharide pair can be designed, screened or selected according to the methods known in the art including the methods disclosed in Kuo et al., *J. Immunol. Methods*, 20 43(1):35-47 (1981); and Brandt et al., *J. Immunol.*, 108(4):913-20 (1972) (a radioactive antigen-binding assay for *Neisseria meningitidis* polysaccharide antibody). Kuo et al., *J. Immunol. Methods*, 43(1):35-47 (1981) provides a polyethylene glycol (PEG) radioimmunoprecipitation assay for the detection of antibody to *Haemophilus influenzae* b capsular 25 polysaccharide, polyribosyribitol phosphate (PRP). The radioactive antigen, [<sup>3</sup>H]PRP, with a high specific activity, was produced by growing the organism in the presence of [<sup>3</sup>H]ribose and was purified by hydroxylapatite and Sepharose™ 4B column chromatography. In the assay, PEG (12.5%) was used to separate antibody-bound [<sup>3</sup>H]PRP from 30 free [<sup>3</sup>H]PRP. The assay covered the range of 0.5 and 20 ng antibody/assay at a maximum sensitivity of 0.5 approximately 1.0 ng

antibody/assay. With various dilutions (1-20 ng antibody/assay) of S. Klein reference antiserum, the within-run coefficient of variation (CV) of 10 replicates ranged from 3.5 to 8.5%. Average CVs of 8.9% and 11.0% were obtained in the between-run and day-to-day reproducibility 5 studies. The binding of [<sup>3</sup>H]PRP to S. Klein reference antiserum was severely inhibited by a minute amount of non-radioactive PRP; however, no significant interference was found in the presence of high concentrations of polysaccharides from *Escherichia coli* K100 and *Streptococcus pneumoniae* indicating that the RIA was highly specific for 10 antibody to *H. influenza* b PRP.

In addition, known protein/polysaccharide binding pairs can be used in the methods and with the products provided herein (see, e.g., Yamaguchi, et al., *Oral Microbiol. Immunol.*, 13(6):348-54 (1998) (capsule-like serotype-specific polysaccharide antigen/lipopolysaccharide 15 from *Actinobacillus actinomycetemcomitans*/human complement-derived opsonins); Lucas, et al., *J. Immunol.*, 161(7):3776-80 (1998) (kappa II-A2 light chain CDR-3 junctional residues in human antibody/*Haemophilus influenza* type b polysaccharide); Miller, et al., *Carbohydr. Res.*, 309(3):219-26 (1998) (fragments of the *Shigella dysenteriae* type 1 O- 20 specific polysaccharide/monoclonal IgM 3707 E9); Prehm, et al., *Protein Expr. Purif.*, 7(4):343-6 (1996) (digitonin/hyaluronate synthase); Jiang, et al., *Infect. Immun.*, 63(7):2537-40 (1995) (mannose-binding 25 protein/*Klebsiella O3* lipopolysaccharide); Pelkonen, et al., *J. Bacteriol.*, 174(23):7757-61 (1992) (bacteriophage depolymerase/bacterial polysaccharide); Morishita, et al., *Biochem. Biophys. Res. Commun.*, 176(3):949-57 (1991) (Microbial polysaccharide, HS-142-1/guanylyl 30 cyclase-containing receptor); Ohtomo, et al., *Can. J. Microbiol.*, 36(3):206-10 (1990) (staphylococcal cell surface polysaccharide/human fibrinogen); Yamagishi, et al., *FEBS Lett.*, 225(1-2):109-12 (1987) (heparin or dermatan sulfate/thrombin); DeAngelis, et al., *J. Biol. Chem.*, 262(29):13946-52 (1987) (sulfated fucans/bindin, the adhesive protein

from sea urchin sperm); Volanakis, et al., *Mol. Immunol.*, 20(11):1201-7 (1983) (human C4/C-reactive protein-pneumococcal C-polysaccharide complexes); Naruse, et al., *J. Biochem. (Tokyo)*, 90(3):581-7 (1981) (a polysaccharide from the cortex of sea urchin egg/microtubule-associated proteins); Levy, et al., *J. Exp. Med.*, 153(4):883-96 (1981) (agaropectin and heparin/human IgG proteins); Hu, et al., *Biochemistry*, 14(10):2224-30 (1975) (glycogen phosphorylase A/a series of semisynthetic, branched saccharides); Fagerstrom, *Microbiology*, 140(9):2399-407 (1994) (raw-starch-binding consensus amino acids in the C-terminal part of 5 glucoamylase P); Murata, et al., *J. Vet. Med. Sci.*, 57(3):419-25 (1995) (C-polysaccharide/C-reactive protein (CRP)); Reason, et al., *Infect. 10 Immun.*, 67(2):994-7 (1999) (Antibodies having light (L) chains encoded by the kappa-I-A2 variable region/Haemophilus influenza type b polysaccharide (Hib PS)).

15           h.     Metal binding moieties

The conjugate can contain a metal binding moiety, such as a metal binding protein, peptide or effective fragment thereof. The specific binding partner can be metal ions generally, a set of metal ions or a particular metal ion. Any metal binding moiety is contemplated. For 20 example, the metal binding sequence can bind to a sodium, a potassium, a magnesium, a calcium, a chlorine, an iron, a copper, a zinc, a manganese, a cobalt, an iodine, a molybdenum, a vanadium, a nickel, a chromium, a fluorine, a silicon, a tin, a boron or an arsenic ion.

Any metal binding moiety/metal ion pair can be designed, screened 25 or selected according to the methods known in the art including the methods disclosed in U.S. Patent No. 5,679,548; Kang et al., *Virus Res.*, 49(2):147-54 (1997); Dealwis et al., *Biochemistry*, 34(43):13967-73 (1995); and Hutchens et al., *J. Chromatogr.*, 604(1):125-32 (1992).

U.S. Patent No. 5,679,548 discloses a method for producing a 30 metal binding site in a polypeptide capable of binding a preselected metal ion-containing molecule, the step of inducing mutagenesis of a

complementarity determining region (CDR) of an immunoglobulin heavy or light chain gene, where mutagenesis introduces a metal binding site, by amplifying the CDR of the gene by a primer extension reaction using a primer oligonucleotide, the oligonucleotide comprising: a) a 3' terminus and a 5' terminus comprising; b) a nucleotide sequence at the 3' terminus complementary to a first framework region of the heavy or light chain immunoglobulin gene; c) a nucleotide sequence at the 5' terminus complementary to a second framework region of the heavy or light chain immunoglobulin gene; and d) a nucleotide sequence between the 3' terminus and 5' terminus according to the formula; [NNS]<sub>a</sub>, wherein N is independently any nucleotide, S is G or C, and a is from 3 to about 50, and the 3' and 5' terminal nucleotide sequences having a length of about 6 to 50 nucleotides, and sequences complementary thereto.

U.S. Patent No. 5,679,548 also describes a method for producing a metal binding site in a polypeptide capable of binding a preselected metal ion-containing molecule, the step of inducing mutagenesis of a complementarity determining region (CDR) of an immunoglobulin heavy or light chain gene by amplifying the CDR of the gene by a primer extension reaction using a primer oligonucleotide, the oligonucleotide comprising: a) a 3' terminus and a 5' terminus; b) a nucleotide sequence at the 3' terminus complementary to a first framework region of the heavy or light chain immunoglobulin gene; c) a nucleotide sequence at the 5' terminus complementary to a second framework region of the heavy or light chain immunoglobulin gene; and d) a nucleotide sequence between 3' terminus and 5' terminus according to the formula: -X-[NNK]<sub>a</sub>-X-[NNK]-X, wherein N is independently any nucleotide, K is G or T, X is a trinucleotide encoding a native amino acid residue coded by the immunoglobulin gene and a is from 3 to about 50, and the 3' and 5' terminal nucleotide sequences having a length of about 6 to 50 nucleotides, and sequences complementary thereto. Preferably, the immunoglobulin to be mutagenized is a human immunoglobulin, the CDR is CDR3, the

mutagenizing oligonucleotide has the formula:

5'-GTGTATTATTGTGCGAGA(NNS)<sub>x</sub>TGGGGCCAAGGGACCACG-3' (SEQ ID No. 24), and the preselected metal ion-containing molecule is magnetite, copper(II), zinc(II), lead(II), cerium(III), or iron(III).

5 Kang et al., *Virus Res.*, 49(2):147-54 (1997) isolated human papillomavirus (HPV) type 18 E7 gene by polymerase chain reaction (PCR) amplification from tissues of Korean cervical cancer patients and cloned into a plasmid vector, pET-3a, for the expression of recombinant E7 protein (rE7) in *Escherichia coli*. The rE7 protein was purified to the

10 homogeneity and its purity was confirmed by HPLC. The purified protein was analyzed for the metal-binding properties by UV spectroscopy and it was shown that two Cd<sup>2+</sup> or Zn<sup>2+</sup> ions bind to one E7 protein by the metal-sulfur ligand formation via two Cys-X-X-Cys motifs in E7 protein. When the change of intrinsic fluorescence of tryptophan residue was

15 analyzed for rE7-Zn complex, the blue shift of emission wavelength and the decrease in maximum intensity of emission were observed compared with rE7. These results suggest that Zn<sup>2+</sup>-bound rE7 has undergone conformational change, in which a tryptophan residue located in the second Cys-X-X-Cys motif was moved into solvent-inaccessible or

20 hydrophobic environment.

Dealwis et al., *Biochemistry*, 34(43):13967-73 (1995) present the refined crystal structures of three different conformational states of the Asp153-->Gly mutant (D153G) of alkaline phosphatase (AP), a metalloenzyme from *Escherichia coli*. The apo state is induced in the

25 crystal over a 3 month period by metal depletion of the holoenzyme crystals. Subsequently, the metals are reintroduced in the crystalline state in a time-dependent reversible manner without physically damaging the crystals. Two structural intermediates of the holo form based on data from a 2 week (intermediate I) and a 2 month soak (intermediate II) of the

30 apo crystals with Mg<sup>2+</sup> and Zn<sup>2+</sup> have been identified. The three-dimensional crystal structures of the apo (R = 18.1%),

intermediate I ( $R = 19.5\%$ ), and intermediate II ( $R = 19.9\%$ ) of the D153G enzyme have been refined and the corresponding structures analyzed and compared. Large conformational changes that extend from the mutant active site to surface loops, located 20 Å away, are observed

5 in the apo structure with respect to the holo structure. The structure of intermediate I shows the recovery of the entire enzyme to an almost native-like conformation, with the exception of residues Asp 51 and Asp 369 in the active site and the surface loop (406-410) which remains partially disordered. In the three-dimensional structure of intermediate II,

10 Asp 51 and Asp 369 are essentially in a native-like conformation, but the main chain of residues 406-408 within the loop is still not fully ordered. The D153G mutant protein exhibits weak, reversible, time dependent metal binding in solution and in the crystalline state.

Hutchens et al., *J. Chromatogr.*, 604(1):125-32 (1992) prepared

15 synthetic peptides representing metal-binding protein surface domains from the human plasma metal transport protein known as histidine-rich glycoprotein (HRG) to evaluate biologically relevant peptide-metal ion interactions. Three synthetic peptides, representing multiples of a 5-residue repeat sequence (Gly-His-His-Pro-His) (SEQ ID No. 25) from

20 within the histidine- and proline-rich region of the C-terminal domain were prepared. Prior to immobilization, the synthetic peptides were evaluated for identity and sample homogeneity by matrix-assisted UV laser desorption time-of-flight mass spectrometry (LDTOF-MS). Peptides with bound sodium and potassium ions were observed; however, these signal

25 intensities were reduced by immersion of the sample probe tip in water. Mixtures of the three different synthetic peptides were also evaluated by LDTOF-MS after their elution through a special immobilized peptide-metal ion column designed to investigate metal ion transfer. It was found that LDTOF-MS to be a useful new method to verify the presence of

30 peptide-bound metal ions.

In addition, the protein/metal binding pairs, which are known (see,

e.g., DiDonato, et al., *Adv. Exp. Med. Biol.*, 448:165-73 (1999) (copper/copper binding domain from the Wilson disease copper transporting ATPase (ATP7B)); Buchko, et al., *Biochem Biophys. Res. Commun.*, 254(1):109-13 (1999) (Zn<sup>2+</sup>/Xenopus laevis nucleotide excision repair protein XPA); Lai, et al., *Biochemistry*, 37(48):7005-15 (1998) (Zn<sup>2+</sup>/hdm2 RING finger domain); Mitterauer, et al., *Biochemistry*, 37(46):16183-91 (1998) (The C2 catalytic domain of adenylyl cyclase contains the second metal ion (Mn<sup>2+</sup>) binding site); Hess, et al., *Protein Sci.*, 7(9):1970-5 (1998) (Zn<sup>2+</sup>/Human nucleotide excision repair protein XPA); Goedken, et al., *Proteins*, 33(1):135-43 (1998) (Mg<sup>2+</sup> and Mn<sup>2+</sup>/ribonuclease H domain of Moloney murine leukemia virus reverse transcriptase); Chang, et al., *Protein Eng.*, 11(1):41-6 (1998) (beta-domain of metallothionein); Champeil, et al., *J. Biol. Chem.*, 273(12):6619-31 (1998) (cytosolic portion of sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase); Bavoso, et al., *Biochem. Biophys. Res. Commun.*, 242(2):385-9 (1998) (zinc finger peptide containing the Cys-X2-Cys-X4-His-X4-Cys domain encoded by the Drosophila Fw-element); Gitschier, et al., *Nat. Struct. Biol.*, 5(1):47-54 (1998) (metal-binding domain from the Menkes copper-transporting ATPase); Gadhavi, *FEBS Lett.*, 417(1):145-9 (1997) (Zn<sup>2+</sup>/ion binding site in the DNA binding domain of the yeast transcriptional activator GAL4); Roehm, et al., *Biochemistry*, 36(33):10240-5 (1997) (Zn<sup>2+</sup>/RING finger domain of BRCA1); Dalton, et al., *Mol. Cell Biol.*, 17(5):2781-9 (1997) (metal response element-binding transcription factor 1 DNA binding involves zinc interaction with the zinc finger domain); Essen, et al., *Biochemistry*, 36(10):2753-62 (1997) (Ca<sup>2+</sup>/A ternary metal binding site in the C2 domain of phosphoinositide-specific phospholipase C-delta1); Curtis, et al., *EMBO J.*, 16(4):834:43 (1997) (Zn<sup>2+</sup>/CCHC metal-binding domain in Nanos); Worthington, et al., *Proc. Natl. Acad. Sci. USA*, 93(24):13754-9 (1996) (zinc-binding domain of Nup475); Mahadevan, et al., *Biochemistry*, 34(7):2095-106 (1995) (Ba<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>/A divalent metal ion binding site in

the kinase insert domain of the alpha-platelet-derived growth factor receptor); Pan, et al., *Biochem. Biophys. Res. Commun.*, 202(1):621-8 (1994) (alpha and beta domains of mammalian metallothionein); Borden, et al., *FEBS Lett.*, 335(2):255-60 (1993) ( $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ /cysteine/histidine-rich metal binding domain from Xenopus nuclear factor XNF7); Chauhan, et al., *J. Bacteriol.*, 175(22):7222-7 (1993) ( $\text{Mg}^{2+}$ /Bradyrhizobium japonicum delta-aminolevulinic acid dehydratase is metal-binding domain); Knegtel, et al., *Biochem. Biophys. Res. Commun.*, 192(2):492-8 (1993) ( $\text{Zn}^{2+}$ /metal coordination in the human retinoic acid receptor-beta DNA binding domain); Spencer, et al., *Biochem. J.*, 290(1):279-87 (1993) ( $\text{Co}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ /5-aminolevulinic acid dehydratase from Escherichia coli reactive thiols at the metal-binding domain); Mau, et al., *Protein Sci.*, 1(11):1403-12 (1992) ( $\text{Zn}^{2+}$ /GAL4 DNA-binding domain); Vaughan, et al., *Virology*, 189(1):377-84 (1992) ( $\text{Zn}^{2+}$ /The herpes simplex virus immediate early protein ICP27 metal binding domain); Boese, et al., *J. Biol. Chem.*, 266(26):17060-6 (1991) ( $\text{Mg}^{2+}$ /Aminolevulinic acid dehydratase in pea metal-binding domain); Hutchens, et al., *J. Biol. Chem.*, 264(29):17206-12 (1989) ( $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ /DNA-binding estrogen receptor); Stillman, et al., *Biochem. J.*, 262(1):181-8 (1989) ( $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$ /rabbit liver metallothionein 2); Freedman, et al., *Nature*, 334(6182):543-6 (1988) ( $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$ /metal coordination sites within the glucocorticoid receptor DNA binding domain); Stillman, et al., *J. Biol. Chem.*, 263(13):6128-33 (1988) ( $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$ /metallothionein); and Corson, et al., *Biochemistry*, 25(7):1817-26 (1986) ( $\text{Ca}^{2+}$ /calcium-binding proteins C-terminal alpha-helix of a helix-loop-helix metal-binding domain)) can be used in the present system.

Among the preferred pairs, are the following metal binding sequence/metal ion pairs (see, U.S. Patent No. 5,679,548) set forth in the following table.

**Table 6. Examples of Metal Ion Binding Sequence/Metal Ion Pairs**

Metal Ion	Metal Ion Binding Sequence	SEQ ID NO.
5 Mg(II)	SerArgArgSerArgHisHisProArgMetTrpAsnGlyLeuAspVal	26
	GlyArgPheLysArgValArgAspArgTrpValVallePheAspPhe	27
	GlyValAlaArgSerLysLysMetArgGlyLeuTrpArgLeuAspVal	28
	GlyLeuAlaValArgSerLysArgGlyArgPhePheLeuPheAspVal	29
10 Cu(II)	GlyArgValHisHisSerLeuAspVal	30
	SerTrpLysHisHisAlaHisTrpAspVal	31
	GlySerTrpAspHisArgGlyCysAspGly	32
	GlyHisHisMetTyrGlyGlyTrpAspHis	33
15 Mg(II)	GlyHisTrpGlyArgHisSerLeuAspThr	34
	GlyHisIleLeuHisHisGlnLeuAspLeu	35
	SerSerGlnArgLeuMetLeuGlyAspAsn	36
	SerHisHisGlyHisHisTyrLeuAsnHis	37
20 Zn(II)	GlyLysLeuMetMetSerTrpCysArgAspThrGluGlyCysAspHis	38
	GlyAspThrHisArgGlyHisLeuArgHisHisLeuProHisAspTrp	39
	GlyTrpGlyLeuTrpMetLysProPheValTrpArgAlaTrpAspMet	40
	GlyArgValHisHisSerLeuAspVal	41
25 Pb(III)	SerHisThrHisAlaLeuProLeuAspPhe	42
	GlyGlnSerSerGlyGlyAspThrAspAsp	43
	GlyGlnTrpThrProArgGlyAspAspPhe	44
	GlyArgCysCysProSerSerCysAspGlu	45
30 Ce(III)	GlyProAlaLysHisArgHisArgHisValGlyGlnMetHisAspSer	46
	GlyAsnLeuArgArgLysThrSerAspIle	47
	GlyGluSerAspSerLysArgGluAspGly	48
	GlyGlyProSerLeuAlaValGlyAspTrp	49
35 Ce(III)	GlyProLeuGlnHisThrTyrProAspTyr	50
	GlyTrpLysValThrAlaGluAspSerThrGluGlyLeuPheAspLeu	51
	GlyThrArgValTrpArgValCysGlnTrpAsnHisGluGluAspGly	52
	GlyGluTrpTrpCysSerPheAlaMetCysProAlaArgTrpAspPhe	53
40 Ce(III)	GlyAspThrIlePheGlyValThrMetGlyTyrTyrAlaMetAspVal	54
	GlyGlnValMetGlnGluLeuGlyAspAla	55
	GlyLeuThrGluGlnGlnLeuGlnAspGly	56
	GlyTyrSerTyrSerValSerProAspAla	57
45 Ce(III)	GlyArgLeuGlyLeuValMetThrAspGlu	58
	SerThrTrpProGlyArgGlnArgLeuGlyGlnAlaLeuSerAspSer	59
	GlyTyrGluLeuSerTrpGlyValAspGlnGlnGluTrpTrpAspIle	60
	GlyProValArgGlyLeuAspGlnSerLysGlyValArgTyrAspAsn	61
50 Ce(III)	GlyLeuSerGlnHisIleValSerGluThrGlnSerSerGlyAspLeu	62
	GlyLeuGluSerLeuLysValLeuGlyValGlnLeuGlyGlyAspLeu	63
	GlyAsnMetIleLeuGlyGlyProGlyCysTrpSerSerAlaAspIle	64

Metal Ion	Metal Ion Binding Sequence	SEQ ID NO.
Fe(III)	GlyCysTrpAsnValGlnArgLeuValValTyrHisProProAspGly	65
	GlyPheGluValThrCysSerTrpPheGlyHisTrpGlyArgAspSer	66
	SerAlaSerMetArgSerAlaIleGlyLeuTrpArgThrMetAspTyr	67
	GlyAspArgGluIlePheHisMetGinTrpProLeuArgValAspVal	68
	SerGlnAsnProGlnGlnValCysGlyValArgCysGlyGlnAspLys	69
	GlyAsnArgLeuSerSerGlyHisLeuLeuLysGlnGlyGlnAspGly	70
	GlyGlySerAspTrpGlnIleGlyAlaCysCysArgGluAspAspLeu	71
	GlyMetValSerMetMetGlyGlnSerArgProThrGinCysAspCys	72
5	GlyValIleLysTrpIleArgArgTrpValArgThrAlaArgAspVal	73
	GlyTrpPheTrpArgLeuLeuProThrProArgAlaProSerAspVal	74
10	i. Other facilitating agents	
	Facilitating agents can be derived from an enzyme, a transport protein, a nutrient or storage protein, a contractile or motile protein, a structural protein, a defense protein, a regulatory protein, or a fluorescent protein. Exemplary of such other fragments are those derived from an enzyme such as a peroxidase, a urease, an alkaline phosphatase, a luciferase and a glutathione S-transferase.	
	1) Peroxidase	
	20 Any peroxidase can be used in the present system. More preferably, a horseradish peroxidase is used. For example, the horseradish peroxidases with the following GenBank accession Nos. can be used: E01651; D90116 (prxC3 gene); D90115 (prxC2 gene); J05552 (Synthetic isoenzyme C(HRP-C)); S14268 (neutral); OPRHC (C1 precursor); S00627 (C1C precursor); JH0150 (C3 precursor); S00626 (C1B precursor); JH0149 (C2 precursor); CAA00083 (Armoracia rusticana); and AAA72223 (synthetic horseradish peroxidase isoenzyme C (HRP-C)).	
	2) urease	
	30 Any urease can be used in the present system. For example, the ureases with the following GenBank accession Nos. can be used: AF085729 (Ureaplasma urealyticum serovar); AF056321 (Actinomyces naeslundii); AF095636 (Yersinia pestis); AF006062 (Filobasidiella	

neoformans var. neoformans (URE1)); U81509 (*Coccidioides immitis* urease); AF000579 (*Bordetella bronchiseptica*); U352248 (*Streptococcus salivarius*); U33011 (*Mycobacterium tuberculosis*); U89957 (*Actinobacillus pleuropneumoniae* urease operon (ureABCXEGD));  
5 D14439 (*Thermophilic Bacillus*); L40490 (*Ureaplasma urealyticum* T960 urease); L40489 (*Ureaplasma urealyticum* strain 7); U40842 (*Yersinia pseudotuberculosis*); M65260 (*Canavalia ensiformis*); U29368 (*Bacillus pasteurii* urease operon); L25079 (*Helicobacter heilmannii* urease); L24101 (*Yersinia enterocolitica*); M31834 (*P.mirabilis* urease operon); M36068  
10 (K.aerogenes); L07039 (*Klebsiella pneumoniae*); M60398 (*H.pylori*); L03308 (*E.coli* urease gene cluster); L03307 (*E.coli* urease gene cluster).

### 3) Alkaline phosphatase

Any alkaline phosphatase can be used in the present system. For example, the alkaline phosphatases encoded by nucleic acids with the following GenBank accession Nos. can be used: AB013386 (*Bombyx mori* s-Alp soluble alkaline phosphatase); AF154110 (*Enterococcus faecalis* (phoZ)); M13077 (Human placental); AF052227 (*Bos taurus* intestinal); AF052226 (*Bos taurus* intestinal); AF079878 (*Thermus* sp. (TAP)); AF047381 (*Pseudomonas aeruginosa* (phoA)); U49060 (*Bacillus subtilis* (phoD)); J03930 (Human intestinal (ALPI)); J03252 (Human alkaline (ALPP)); U19108 (Gallus tissue-nonspecific); M13345 (*E. coli*); U31569 (*Felis catus* (alpl)); L36230 (*Zymomonas mobilis* (phoD)); M19159 (Human placental heat-stable (PLAP-1)); M12551 (Human placental (PLAP)); M31008 (Human intestinal); J04948 (Human (ALP-1));  
25 J03572 (Rat); M61705 (Mouse intestinal (IAP)); M61704 (Mouse embryonic); M61706 (Mouse (AP) pseudogene); M21134 (*S.cerevisiae* (rALPase)); L07733 (Cow intestinal (IAP)); M18443 (Bovine); M77507 (*Synechococcus* sp. atypical); M33965 (*S.marcescens* (phoA)); M33966 (*E.fergusonii* (phoA)); M29670 (*E.coli* (phoA)); M29669 (*E.coli* (phoA));  
30 M29668 (*E.coli* (phoA)); M29667 (*E.coli* (phoA)); M29666 (*E.coli* (phoA)); M29665 (*E.coli* (phoA)); M29664 (*E.coli* (phoA)); M29663

**MISSING AT THE TIME OF PUBLICATION**

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Enhancer firefly luciferase (luc+) gene); U47296 (Cloning vector pGL3-  
Control firefly luciferase (luc+) gene); U47295 (Cloning vector pGL3-  
Basic firefly luciferase (luc+) gene); U47123 (Cloning vector pSP-  
luc+NF, luciferase cassette fusion vector); U47122 (Cloning vector pSP-  
5 luc+, Luciferase cassette vector); M10961 (*V.harveyi* (luxA and luxB);  
M65067 (*Photobacterium phosphoreum* (luxA and luxB); M62917  
(*Xenorhabdus luminescens* (luxA, luxB, luxC, and luxD); M25666  
(*V.hilgendorfii*); M63501 (*Renilla reniformis*); M15077 (*P.pyralis* (firefly));  
M26194 (*Luciola cruciata*); M55977 (*X.luminescens* (luxA and luxB));  
10 M90093 (*Xenorhabdus luminescens* (luxA) and (luxB) (luxE)); U03687  
(*Photinus pyralis* modified luciferase gene).

##### 5) Glutathione S-transferase

A glutathione S-transferase (GST), more preferably a *Schistosoma japonicum* glutathione S-transferase, can be included in the conjugate.

15 GST occurs naturally as a 26 kDa protein which can be expressed in *E. coli* with full enzymatic activity. Conjugates that contain the full length GST also demonstrate GST enzymatic activity and can undergo dimerization as observed in nature (Parker et al., *J. Mol. Biol.*, 213:221 (1990); Ji, et al., *Biochemistry*, 31:10169 (1992); and Maru et al., *J. 20 Biol. Chem.*, 271:15353 (1996)). The crystal structure of recombinant *Schistosoma japonicum* GST from pGEX vectors has been determined (McTigue et al., *J. Mol. Biol.*, 246:21 (1995)) and matches that of the native protein. Conjugates that contain a GST can be readily purified.

For example, fusion proteins are easily purified from bacterial lysates by affinity chromatography using Glutathione Sepharose 4B contained in the GST Purification Modules (Amersham Pharmacia Biotech, Inc.). Cleavage of the desired protein from GST is achieved using a site-specific protease whose recognition sequence is located immediately upstream from the multiple cloning site on the pGEX plasmids. Fusion 25 proteins can be detected using a colorimetric assay or immunoassay provided in the GST Detection Module, or by Western blotting with anti-

GST antibody. The system has been used successfully in many applications such as molecular immunology (Toye et al., *Infect. Immun.*, 58:3909 (1990)), the production of vaccines (Fikrig et al., *Science*, 250:553 (1990); and Johnson et al., *Nature*, 338:585 (1989)) and 5 studies involving protein-protein (Kaelin et al., *Cell*, 64:521 (1991)) and DNA-protein (Kaelin et al., *Cell*, 65:1073 (1991)) interactions.

Any glutathione S-transferase is contemplated. For example, the glutathione S-transferase encoded by nucleic acid with the following GenBank accession Nos. can be used: [AF112567], *Fasciola gigantica*; 10 [M77682], *Fasciola hepatica*; [AB016426], *Cavia porcellus*; [AF144382], *Arabidopsis thaliana*; [AF133251], *Gallus*; [AB021655], *Issatchenkovia orientalis*; [AF133268], *Manduca sexta*; [AF125273], *Homo sapiens* tissue-type skeletal muscle; [AF125271], *Homo sapiens* tissue-type pancreas; [AB026292], *Sphingomonas paucimobilis*; [AB026119], 15 [U49179], *Bos taurus*; [AF106661], *Rattus norvegicus* (GstYb4); [L15387], *Gallus class-alpha*; [AF051318], *Clonorchis sinensis*; [AF101269], *Echinococcus granulosus*; [AF077609], *Boophilus microplus*; [AA956087], *Homo sapiens* microsomal; [AF004358], *Aegilops squarrosa*; [AF109714], *Triticum aestivum*; 20 [U86635], *Rattus norvegicus* glutathione; [AF111428], *Drosophila melanogaster* microsomal; [AF111426], *Drosophila melanogaster* microsomal; [AF071163], *Anopheles gambiae*; [AF071162], *Anopheles gambiae*; [AF071161], *Anopheles gambiae*; [AF071160], *Anopheles gambiae*; [D10524], *Nicotiana tabacum*; [AF062403], *Oryza sativa*; 25 [U77604], *Homo sapiens* microsomal (MGST2); [U30897], Human (P1b); [U62589], Human (GSTp1c); [U42463], *Coccomyxa sp. PA*; [AF001779], *Sphingomonas paucimobilis* strain epa505; [U51165], *Cycloclasticus oligotrophus* (XYLK); [AF025887], *Homo sapiens* (GSTA4); [U66342], *Plutella xylostella*; [AF051238], *Picea mariana* (Sb52); [AF051214], *Picea mariana* (Sb18); [AF079511], 30 *Mesembryanthemum crystallinum* clone R6-R37; [D10026], *Rattus*

norvegicus Yrs-Yrs; [AF048978], Glycine max 2,4-D inducible (GST<sub>a</sub>); [AF043105], Homo sapiens (GSTM3); [AF057172], Homo sapiens (GSTT2P); [U21689], Human; [AH006027], Homo sapiens (GSTT2); [AF057176], Homo sapiens (GSTT2); [AF050102], Oryza sativa (GST1);  
5 [AF044411], Schistosoma japonicum; [U87958], Culicoides variipennis (CVGST1); [AF026977], Homo sapiens microsomal (MGST3); [AF027740], Homo sapiens microsomal (MGST1L1); [AF005928], Echinococcus granulosus; [AF001103], Pseudomonas (phnC); [AF010241], Caenorhabditis elegans (CeGST3); [AF010240],  
10 Caenorhabditis elegans (CeGST2); [AF010239], Caenorhabditis elegans (CeGST1); [AF002692], Solanum commersonii (GST1); [L38503], Homo sapiens (GSTT2); [M97937], E. coli/S. japonicum; [L29427], Rat GST-P gene; [M14654], Schistosoma japonicum Sj26 antigen; [AB000884], Sus scrofa; [D44465], Arabidopsis thaliana; [D17673], Arabidopsis thaliana;  
15 [D17672], Arabidopsis thaliana; [U78784], Anopheles dirus; [U71213], Human microsomal; [U70672], Arabidopsis thaliana; [U24428], Mus musculus; [U43126], Naegleria fowleri; [X14233], D.melanogaster (GST); [L32092], Manduca sexta; [L32091], Manduca sexta; [U30489], Arabidopsis thaliana; [M24889], Artificial maize; [L05915], Dianthus  
20 caryophyllus; [M15872], Human; [L23766], Oryctolagus cuniculus; [J03679], Solanum tuberosum; [U12472], Human (GST phi); [U15654], Mus musculus; [M24485], Homo sapiens (GSTP1); [L28771], Onchocerca volvulus; [M14777], Human; [M16594], Human; [M21758], Human; [J03914], Rat; [K01932], Rat liver; [J02810], Rat prostate;  
25 [M25891], Rat; [M11719], Rat liver; [M28241], Rat; [J03752], Rat; [M73483], Mouse (GST Yc); [J04696], Mouse (GST5-5); [J04632], Mouse (GST1-1); [M59772], M.auratus; [L20466], Chinese hamster; [M25627], Human liver; [J03746], Human (SEQ ID No. 75); [M16901], Maize; [M64268], Dianthus caryophyllus; [L11601], Arabidopsis thaliana;  
30 [L07589], Arabidopsis thaliana; [M74529], Oryctolagus cuniculus; [M74528], Oryctolagus cuniculus; [M98271], Schistosoma mansoni 28

kDa; [L23126], *Lucilia cuprina*; [M95198], *Drosophila melanogaster*; [L26544], *Methylophilus* sp.; [U14753], *Dirofilaria immitis*; [U12679], *Zea mays*; [L02321], Human (GSTM5); [L15386], Chicken.

In addition, commercially available Glutathione S-transferase (GST) gene fusion system can be used. For example, the Glutathione S-transferase (GST) Gene Fusion System (Amersham Pharmacia Biotech, Inc.) can be used. The system from Amersham Pharmacia Biotech, Inc. is an integrated system for the expression, purification and detection of fusion proteins produced in *E. coli*. The system includes three primary components: pGEX plasmid vectors, various options for GST purification and a variety of GST detection products. A series of site-specific proteases complements the system. The pGEX plasmids are designed for inducible, high-level intracellular expression of genes or gene fragments as fusions with *Schistosoma japonicum* GST (Smith and Johnson, *Gene*, 17:31 (1988)). All pGEX Vectors (GST Gene fusion) offer: 1) A tac promoter for chemically inducible, high-level expression; 2) an internal *lac Z* gene for use in any *E. coli* host; 3) very mild elution conditions for release of fusion proteins from the affinity matrix, thus minimizing effects on antigenicity and functional activity; and 4) PreScission, thrombin or factor Xa protease recognition sites for cleaving the desired protein from the fusion product.

The GST Detection Module from Amersham Pharmacia Biotech, Inc. can be used for identification of GST fusion proteins using either a biochemical or immunological assay. In the biochemical assay, glutathione and 1-chloro-2-4-dinitrobenzene (CDNB) serve as substrates for GST to yield a yellow product detectable at 340 nm (Habig et al., *J. Biol. Chem.*, 249:7130 (1974)). An affinity-purified goat anti-GST polyclonal antibody suitable for Western blots is used in the immunoassay.

The GST 96-Well Detection Module from Amersham Pharmacia Biotech, Inc. contains five microtiter strip plates, horseradish peroxidase

(HRP) conjugated anti-GST antibody and recombinant GST protein. The wells of each plate are coated with purified anti-GST antibody to capture GST fusion proteins and are preblocked to provide a low background. HRP conjugated antibody enables sensitive detection of GST proteins.

5       The anti-GST antibody supplied in the system from Amersham Pharmacia Biotech, Inc. is a polyclonal antibody purified from the sera of goats immunized with purified *schistosomal* glutathione S-transferase (GST). Because of its polyclonal nature, it can recognize more than one epitope on GST, thereby improving its capacity for recognizing GST

10      fusion proteins even if some binding sites are masked due to recombinant protein folding.

Factor Xa can be used for site-specific separation of the GST affinity tag from proteins expressed using pGEX X vectors. Factor Xa enables the site-specific cleavage of fusion proteins containing an accessible Factor Xa recognition sequence. It can be used either following affinity purification or while fusion proteins are bound to Glutathione Sepharose 4B. Factor Xa, purified from bovine plasma, is used to digest fusion proteins prepared from pGEX vectors containing the recognition sequence for factor Xa (pGEX-3X, pGEX-5X-1, pGEX-5X-2 and pGEX-5X-3). It specifically cleaves following the tetrapeptide Ile-Glu-Gly-Arg (SEQ ID No. 77) (Nagai and Thøgersen, *Nature*, 309:810 (1984); and Nagai and Thøgersen, *Methods Enzymol.*, 153:461 (1987)). In the system from Amersham Pharmacia Biotech, Inc., one unit of Factor Xa cleaves  $\geq$  90% of 100  $\mu$ g of a test GST fusion protein when incubated in 1 mM CaCl<sub>2</sub>, 100 mM NaCl and 50 mM Tris-HCl (pH 8.0) at 22°C for 16 hours.

PreScission protease can be used for site-specific separation of the GST affinity tag from proteins expressed using pGEX-6P vectors. It enables the low-temperature cleavage of fusion proteins containing the PreScission Protease recognition sequence. It can be used either following affinity purification or while fusion proteins are bound to

Glutathione Sepharose 4B. PreScission Protease is a genetically engineered fusion protein containing human rhinovirus 3C protease and GST (Walker et al., *Bio/Technology*, 12:601 (1994)). This protease was specifically designed to facilitate removal of the protease by allowing

5 simultaneous protease immobilization and cleavage of GST fusion proteins produced from pGEX-6P vectors (pGEX-6P-1, pGEX-6P-2, and pGEX-6P-3). PreScission Protease specifically cleaves between the Gln and Gly residues of the recognition sequence of

LeuGluValLeuPheGln/GlyPro (SEQ ID No. 78) (Cordingley et al., *J. Bio.*

10 *Chem.*, 265:9062 (1990)). In the system from Amersham Pharmacia Biotech, Inc., one unit of PreScission protease will cleave  $\geq$  90% of 100  $\mu$ g of a test GST-fusion protein in 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.0 at 5°C for 16 hours.

Thrombin can be used for site-specific separation of the GST

15 affinity tag from proteins expressed using pGEX T vectors. It enables the site-specific cleavage of fusion proteins containing an accessible thrombin recognition sequence. It is purified from bovine plasma; functionally free of other clotting factors, plasminogen and plasmin. It can be used either following affinity purification or while fusion proteins are bound to

20 Glutathione Sepharose 4B. Thrombin is used to digest fusion proteins prepared from pGEX vectors containing the recognition sequence for thrombin (pGEX-1T, pGEX-2T, pGEX-2TK, pGEX-4T-1, pGEX-4T2 and pGEX-4T-3). In the system from Amersham Pharmacia Biotech, Inc., one unit of Thrombin cleaves  $\geq$  90% of 100  $\mu$ g of a test GST fusion protein

25 when incubated in 1x PBS at 22°C for 16 hours.

#### 6) Defense proteins

The conjugates can contain defense protein, such as an antibody. Any antibody, including polyclonal, monoclonal, single chain or Fab fragments, can be used.

### 7) Fluorescent moieties

The conjugates can contain a fluorescent moiety, such as a green, a blue or a red fluorescent protein. Any green, blue or red fluorescent protein can be used in the present system. For instance, the green 5 fluorescent proteins encoded by nucleic acids with the following GenBank accession Nos. can be used: U47949 (AGP1); U43284; AF007834 (GFPuv); U89686 (*Saccharomyces cerevisiae* synthetic green fluorescent protein (cox3::GFPm-3) gene); U89685 (*Saccharomyces cerevisiae* synthetic green fluorescent protein (cox3::GFPm) gene); U87974 10 (Synthetic construct modified green fluorescent protein GFP5-ER (mgfp5-ER)); U87973 (Synthetic construct modified green fluorescent protein GFP5 (mgfp5)); U87625 (Synthetic construct modified green fluorescent protein GFP-ER (mgfp4-ER)); U87624 (Synthetic construct green fluorescent protein (mgfp4) mRNA)); U73901 (*Aequorea victoria* mutant 15 3); U50963 (Synthetic); U70495 (soluble-modified green fluorescent protein (smGFP)); U57609 (enhanced green fluorescent protein gene); U57608 (enhanced green fluorescent protein gene); U57607 (enhanced green fluorescent protein gene); U57606 (enhanced green fluorescent protein gene); U55763 (enhanced green fluorescent protein (egfp)); 20 U55762 (enhanced green fluorescent protein (egfp)); U55761 (enhanced green fluorescent protein (egfp)); U54830 (Synthetic *E. coli* Tn3-derived transposon green fluorescent protein (GF)); U36202; U36201; U19282; U19279; U19277; U19276; U19281; U19280; U19278; L29345 (*Aequorea victoria*); M62654 (*Aequorea victoria*); M62653 (*Aequorea 25 victoria*); AAB47853 ((U87625) synthetic construct modified green fluorescent protein (GFP-ER)); AAB47852 ((U87624) synthetic construct green fluorescent protein).

Similarly, the blue fluorescent proteins encoded by nucleic acids with the following GenBank accession Nos. can be used: U70497 30 (soluble-modified blue fluorescent protein (smBFP); 1BFP (blue variant of green fluorescent protein); AAB16959 (soluble-modified blue fluorescent

protein).

Also similarly, the red fluorescent proteins encoded by nucleic acids with the following GenBank accession Nos. can be used: U70496 (soluble-modified red-shifted green fluorescent protein (smRSGFP);

5 AAB16958 ((U70496) soluble-modified red-shifted green fluorescent protein).

**H. IMMOBILIZATION OF MUTANT DNA REPAIR ENZYMES AND NUCLEIC ACIDS**

10 In the methods for detecting abnormal base-pairings, mutations, and polymorphisms, and the methods for localizing and removing abnormal base-pairings described in Sections B-F, the target nucleic acid strand to be assayed, the reference nucleic acid strand, the target nucleic acid duplex to be assayed, the nucleic acid duplex formed via  
15 hybridization of the target strand and the reference strand, or the mutant DNA repair enzyme or complex thereof can be immobilized on the surface of a support, either directly via a linker. Preferably, the support used is an insoluble support such as a silicon chip. Non-limiting examples of the geometry of the support include beads, pellets, disks, capillaries, hollow  
20 fibers, needles, solid fibers, random shapes, thin films, membranes and chips. Also more preferably, the nucleic acid strand, the nucleic acid duplex or the mutant DNA repair enzyme or complex thereof is immobilized in an array or a well format on the surface.

**1. Immobilization of the mutant DNA repair enzymes**

25 In certain embodiments, where the facilitating agents are designed for linkage to surfaces, recovered, isolated or purified conjugates, such as fusion proteins can be attached to a surface of a matrix material. Immobilization may be effected directly or via a linker. The conjugates may be immobilized on any suitable support, including, but are not limited  
30 to, silicon chips, and other supports described herein and known to those of skill in the art. A plurality of conjugates, which may contain the same or different or a variety of mutant DNA repair enzymes (abnormal base-

pairing trapping enzymes) may be attached to a support, such as an array (*i.e.*, a pattern of two or more) of conjugates on the surface of a silicon chip or other chip for use in high throughput protocols and formats.

It is also noted that the mutant DNA repair enzymes can be linked

5 directly to the surface or via a linker without a facilitating agent linked thereto. Hence, chips containing arrays of mutant DNA repair enzymes are contemplated.

For example, an isolated or purified fusion protein can be attached to the surface as the intact fusion proteins. Alternatively, the protein or

10 peptide fragment portion can be cleaved off and the mutant DNA repair enzyme be attached to the surface. The fusion protein can be cleaved by any methods known in the art such as chemical or enzymatic means.

The cleavage means must be compatible with the linking sequence between the protein or peptide fragment portion and the mutant DNA

15 repair enzyme so that the cleavage is linker sequence specific and the cleaved mutant enzyme is functional, *i.e.*, can be used as a abnormal base-pairing-trapping enzyme. Those skilled in the art can readily determine, if necessary, with empirical studies, which cleavage/linker sequence pair to be used. Many cleavage/linker sequence pairs are well

20 known in the art. For example, Factor Xa can be used for site-specific separation of the GST affinity tag from proteins expressed using pGEX X vectors; PreScission protease can be used for site-specific separation of the GST affinity tag from proteins expressed using pGEX-6P vectors; and Thrombin can be used for site-specific separation of the GST affinity tag

25 from proteins expressed using pGEX T vectors.

The matrix material substrates contemplated herein are generally insoluble materials used to immobilize ligands and other molecules, and are those that are used in many chemical syntheses and separations. Such substrates, also called matrices, are used, for example, in affinity

30 chromatography, in the immobilization of biologically active materials, and during chemical syntheses of biomolecules, including proteins, amino

acids and other organic molecules and polymers. The preparation of and use of matrices is well known to those of skill in this art; there are many such materials and preparations thereof known. For example, naturally-occurring matrix materials, such as agarose and cellulose, may be isolated  
5 from their respective sources, and processed according to known protocols, and synthetic materials may be prepared in accord with known protocols.

The substrate matrices are typically insoluble materials that are solid, porous, deformable, or hard, and have any required structure and  
10 geometry, including, but not limited to: beads, pellets, disks, capillaries, hollow fibers, needles, solid fibers, random shapes, thin films and membranes. Thus, the item may be fabricated from the matrix material or combined with it, such as by coating all or part of the surface or impregnating particles.

15 Typically, when the matrix is particulate, the particles are at least about 10-2000  $\mu\text{M}$ , but may be smaller or larger, depending upon the selected application. Selection of the matrices will be governed, at least in part, by their physical and chemical properties, such as solubility, functional groups, mechanical stability, surface area swelling propensity,  
20 hydrophobic or hydrophilic properties and intended use.

If necessary, the support matrix material can be treated to contain an appropriate reactive moiety. In some cases, the support matrix material already containing the reactive moiety may be obtained commercially. The support matrix material containing the reactive moiety  
25 may thereby serve as the matrix support upon which molecules are linked. Materials containing reactive surface moieties such as amino silane linkages, hydroxyl linkages or carboxysilane linkages may be produced by well established surface chemistry techniques involving silanization reactions, or the like. Examples of these materials are those  
30 having surface silicon oxide moieties, covalently linked to gamma-amino-propylsilane, and other organic moieties; N-[3-(triethoxysilyl)propyl]-

phthalamic acid; and bis-(2-hydroxyethyl)aminopropyltriethoxysilane. Exemplary of readily available materials containing amino group reactive functionalities, include, but are not limited to, para-aminophenyl-triethoxysilane. Also derivatized polystyrenes and other such polymers

5 are well known and readily available to those of skill in this art (e.g., the Tentagel® Resins are available with a multitude of functional groups, and are sold by Rapp Polymere, Tübingen, Germany; see, U.S. Patent No. 4,908,405 and U.S. Patent No. 5,292,814; see, also Butz et al., *Peptide Res.*, 7:20-23 (1994); and Kleine et al., *Immunobiol.*, 190:53-66 (1994)).

10 These matrix materials include any material that can act as a support matrix for attachment of the molecules of interest. Such materials are known to those of skill in this art, and include those that are used as a support matrix. These materials include, but are not limited to, inorganics, natural polymers, and synthetic polymers, including, but are

15 not limited to: cellulose, cellulose derivatives, acrylic resins, glass, silica gels, polystyrene, gelatin, polyvinyl pyrrolidone, co-polymers of vinyl and acrylamide, polystyrene cross-linked with divinylbenzene and others (see, Merrifield, *Biochemistry*, 3:1385-1390 (1964)), polyacrylamides, latex gels, polystyrene, dextran, polyacrylamides, rubber, silicon, plastics,

20 nitrocellulose, celluloses, natural sponges. Of particular interest herein, are highly porous glasses (see, e.g., U.S. Patent No. 4,244,721) and others prepared by mixing a borosilicate, alcohol and water.

Synthetic matrices include, but are not limited to: acrylamides, dextran-derivatives and dextran co-polymers, agarose-polyacrylamide

25 blends, other polymers and co-polymers with various functional groups, methacrylate derivatives and co-polymers, polystyrene and polystyrene copolymers (see, e.g., Merrifield, *Biochemistry*, 3:1385-1390 (1964); Berg et al., in *Innovation Perspect. Solid Phase Synth. Collect. Pap.*, Int. Symp., 1st, Epton, Roger (Ed), pp. 453-459 (1990); Berg et al., *Pept.*,

30 *Proc. Eur. Pept. Symp.*, 20th, Jung, G. et al. (Eds), pp. 196-198 (1989); Berg et al., *J. Am. Chem. Soc.*, 111:8024-8026 (1989); Kent et al., *Isr.*

*J. Chem.*, 17:243-247 (1979); Kent et al., *J. Org. Chem.*, 43:2845-2852 (1978); Mitchell et al., *Tetrahedron Lett.*, 42:3795-3798 (1976); U.S. Patent No. 4,507,230; U.S. Patent No. 4,006,117; and U.S. Patent No. 5,389,449). Methods for preparation of such matrices are well-known to 5 those of skill in this art.

Synthetic matrices include those made from polymers and co-polymers such as polyvinylalcohols, acrylates and acrylic acids such as polyethylene-co-acrylic acid, polyethylene-co-methacrylic acid, polyethylene-co-ethylacrylate, polyethylene-co-methyl acrylate, polypropylene-co-acrylic acid, polypropylene-co-methyl-acrylic acid, polypropylene-co-ethylacrylate, polypropylene-co-methyl acrylate, polyethylene-co-vinyl acetate, polypropylene-co-vinyl acetate, and those containing acid anhydride groups such as polyethylene-co-maleic anhydride, polypropylene-co-maleic anhydride and the like. Liposomes have also been used as solid 10 supports for affinity purifications (Powell et al. *Biotechnol. Bioeng.*, 33:173 (1989)).

For example, U.S. Patent No. 5,403,750, describes the preparation of polyurethane-based polymers. U.S. Pat. No. 4,241,537 describes a plant growth medium containing a hydrophilic polyurethane gel composition prepared from chain-extended polyols; random copolymerization is 20 preferred with up to 50% propylene oxide units so that the prepolymer will be a liquid at room temperature. U.S. Pat. No. 3,939,123 describes lightly crosslinked polyurethane polymers of isocyanate terminated prepolymers containing poly(ethyleneoxy) glycols with up to 35% of a 25 poly(propyleneoxy) glycol or a poly(butyleneoxy) glycol. In producing these polymers, an organic polyamine is used as a crosslinking agent. Other matrices and preparation thereof are described in U.S. Patent Nos. 4,177,038, 4,175,183, 4,439,585, 4,485,227, 4,569,981, 5,092,992, 5,334,640, 5,328,603.

30 U.S. Patent No. 4,162,355 describes a polymer suitable for use in affinity chromatography, which is a polymer of an aminimide and a vinyl

compound having at least one pendant halo-methyl group. An amine ligand, which affords sites for binding in affinity chromatography is coupled to the polymer by reaction with a portion of the pendant halo-methyl groups and the remainder of the pendant halo-methyl groups 5 are reacted with an amine containing a pendant hydrophilic group. A method of coating a substrate with this polymer is also described. An exemplary aminimide is 1,1-dimethyl-1-(2-hydroxyoctyl)amine methacryl-imide and vinyl compound is a chloromethyl styrene.

U.S. Patent No. 4,171,412 describes specific matrices based on 10 hydrophilic polymeric gels, preferably of a macroporous character, which carry covalently bonded D-amino acids or peptides that contain D-amino acid units. The basic support is prepared by copolymerization of hydroxyalkyl esters or hydroxyalkylamides of acrylic and methacrylic acid with crosslinking acrylate or methacrylate comonomers are modified by 15 the reaction with diamines, aminoacids or dicarboxylic acids and the resulting carboxyterminal or aminoterminal groups are condensed with D-analogs of aminoacids or peptides. The peptide containing D-amino-acids also can be synthesized stepwise on the surface of the carrier. For example, U.S. Patent No. 4,178,439 describes a cationic ion exchanger 20 and a method for preparation thereof. U.S. Patent No. 4,180,524 describes chemical syntheses on a silica support.

The fusion protein can be attached to the surface of the matrix material by methods known in the art. Numerous methods have been developed for the immobilization of proteins and other biomolecules onto 25 solid or liquid supports (see, e.g., Mosbach, *Methods in Enzymology*, 44 (1976); Weetall, *Immobilized Enzymes, Antigens, Antibodies, and Peptides*, (1975); Kennedy et al., *Solid Phase Biochemistry, Analytical and Synthetic Aspects*, Scouten, ed., pp. 253-391 (1983); see, generally, *Affinity Techniques. Enzyme Purification: Part B. Methods in Enzymology*, Vol. 34, ed. W. B. Jakoby, M. Wilchek, Acad. Press, N.Y. 30 (1974); and *Immobilized Biochemicals and Affinity Chromatography*,

*Advances in Experimental Medicine and Biology*, vol. 42, ed. R. Dunlap, Plenum Press, N.Y. (1974)).

Among the most commonly used methods are absorption and adsorption or covalent binding to the support, either directly or via a linker,

5 such as the numerous disulfide linkages, thioether bonds, hindered disulfide bonds, and covalent bonds between free reactive groups, such as amine and thiol groups, known to those of skill in art (see, e.g., the PIERCE CATALOG, ImmunoTechnology Catalog & Handbook, 1992-1993, which describes the preparation of and use of such reagents and

10 provides a commercial source for such reagents; Wong, *Chemistry of Protein Conjugation and Cross Linking*, CRC Press (1993); see also DeWitt et al., *Proc. Natl. Acad. Sci. U.S.A.*, 90:6909 (1993); Zuckermann et al., *J. Am. Chem. Soc.*, 114:10646 (1992); Kurth et al., *J. Am. Chem. Soc.*, 116:2661 (1994); Ellman et al., *Proc. Natl. Acad. Sci. U.S.A.*, 91:4708 (1994); Sucholeiki, *Tetrahedron Ltrs.*, 35:7307 (1994); Su-Sun Wang, *J. Org. Chem.*, 41:3258 (1976); Padwa et al., *J. Org. Chem.*, 41:3550 (1971); and Vedejs et al., *J. Org. Chem.*, 49:575 (1984), which describe photosensitive linkers).

To effect immobilization, a composition containing the protein or

20 other biomolecule is contacted with a support material such as alumina, carbon, an ion-exchange resin, cellulose, glass or a ceramic. Fluorocarbon polymers have been used as supports to which biomolecules have been attached by adsorption (see, U.S. Patent No. 3,843,443; Published International PCT Application WO 86/03840).

25 A large variety of methods are known for attaching biological molecules, including proteins and nucleic acids, molecules to solid supports (see e.g., U.S. Patent No. 5451683). For example, U.S. Pat. No. 4,681,870 describes a method for introducing free amino or carboxyl groups onto a silica matrix. These groups may subsequently be

30 covalently linked to other groups, such as a protein or other anti-ligand, in the presence of a carbodiimide. Alternatively, a silica matrix may be

activated by treatment with a cyanogen halide under alkaline conditions. The anti-ligand is covalently attached to the surface upon addition to the activated surface. Another method involves modification of a polymer surface through the successive application of multiple layers of biotin, 5 avidin and extenders (see e.g., U.S. Patent No. 4,282,287). Other methods involve photoactivation in which a polypeptide chain is attached to a solid substrate by incorporating a light-sensitive unnatural amino acid group into the polypeptide chain and exposing the product to low-energy ultraviolet light (see e.g., U.S. Patent No. 4,762,881). Oligonucleotides 10 have also been attached using a photochemically active reagent, such as a psoralen compound, and a coupling agent, which attaches the photoreagent to the substrate (see e.g., U.S. Patent No. 4,542,102 and U.S. Patent No. 4,562,157). Photoactivation of the photoreagent binds a nucleic acid molecule to the substrate to give a surface-bound probe.

15 Covalent binding of the protein or other biomolecule or organic molecule or biological particle to chemically activated solid matrix supports such as glass, synthetic polymers, and cross-linked polysaccharides is a more frequently used immobilization technique. The molecule or biological particle may be directly linked to the matrix support 20 or linked via linker, such as a metal (see, e.g., U.S. Patent No. 4,179,402; and Smith et al., *Methods: A Companion to Methods in Enz.*, 4:73-78 (1992)). An example of this method is the cyanogen bromide activation of polysaccharide supports, such as agarose. The use of perfluorocarbon polymer-based supports for enzyme immobilization and 25 affinity chromatography is described in U.S. Pat. No. 4,885,250. In this method the biomolecule is first modified by reaction with a perfluoroalkylating agent such as perfluoroctylpropylisocyanate described in U.S. Pat. No. 4,954,444. Then, the modified protein is adsorbed onto the fluorocarbon support to effect immobilization.

The activation and use of matrices are well known and may be effected by any such known methods (see, e.g., Hermanson et al., *Immobilized Affinity Ligand Techniques*, Academic Press, Inc., San Diego (1992)). For example, the coupling of the amino acids may be 5 accomplished by techniques familiar to those in the art and provided, for example, in Stewart and Young, *Solid Phase Synthesis*, Second Edition, Pierce Chemical Co., Rockford (1984).

Other suitable methods for linking molecules to solid supports are well known to those of skill in this art (see, e.g., U.S. Patent No. 10 5,416,193). These include linkers that are suitable for chemically linking molecules, such as proteins, to supports and include, but are not limited to, disulfide bonds, thioether bonds, hindered disulfide bonds, and covalent bonds between free reactive groups, such as amine and thiol groups. These bonds can be produced using heterobifunctional reagents 15 to produce reactive thiol groups on one or both of the moieties and then reacting the thiol groups on one moiety with reactive thiol groups or amine groups to which reactive maleimido groups or thiol groups can be attached on the other.

Other linkers include, acid cleavable linkers, such as 20 bismaleimideethoxy propane, acid labile-transferrin conjugates and adipic acid dihydrazide, that would be cleaved in more acidic intracellular compartments; cross linkers that are cleaved upon exposure to UV or visible light and linkers, such as the various domains, such as C<sub>H</sub>1, C<sub>H</sub>2, and C<sub>H</sub>3, from the constant region of human IgG, (Batra et al., *Molecular 25 Immunol.*, 30:379-386 (1993)). Presently preferred linkages are direct linkages effected by adsorbing the molecule to the surface of the matrix.

Other linkages are photocleavable linkages that can be activated by 30 exposure to light (see, e.g., Goldmacher et al., *Bioconj. Chem.*, 3:104-107 (1992)). The photocleavable linker is selected such that the cleaving wavelength does not damage linked moieties. Photocleavable linkers are linkers that are cleaved upon exposure to light (see, e.g., Hazum et al.,

*Pept., Proc. Eur. Pept. Symp.*, 16th, Brunfeldt, K (Ed), pp. 105-110 (1981), which describes the use of a nitrobenzyl group as a photocleavable protective group for cysteine; Yen et al., *Makromol. Chem.*, 190:69-82 (1989), which describes water soluble photocleavable 5 copolymers, including hydroxypropylmethacrylamide copolymer, glycine copolymer, fluorescein copolymer and methylrhodamine copolymer; Goldmacher et al., *Bioconj. Chem.*, 3:104-107 (1992), which describes a cross-linker and reagent that undergoes photolytic degradation upon exposure to near UV light (350 nm); and Senter et al., *Photochem.*

10 *Photobiol.*, 42:231-237 (1985), which describes nitrobenzyloxycarbonyl chloride cross linking reagents that produce photocleavable linkages). The selected linker will depend upon the particular application and, if needed, may be empirically selected.

In a preferred embodiment, the recovered fusion protein is attached 15 to the surface through affinity binding between the protein or peptide fragment of the fusion protein and an affinity binding moiety on the surface.

## 2. Immobilization of nucleic acids

The target nucleic acid strand to be assayed, the reference nucleic 20 acid strand, the target nucleic acid duplex to be assayed, the nucleic acid duplex formed via hybridization of the target strand and the reference strand can be immobilized by any methods known in the art. For example, the immobilization procedures disclosed in the following literatures can be used: Bresser et al., *DNA*, 2(3):243-54 (1983);

25 Hirayama et al., *Nucleic Acids Res.*, 24(20):4098-9 (1996); Kremsky et al., *Nucleic Acids Res.*, 15(7):2891-909 (1987); Macdougall et al., *Biochem. J.*, 191(3):855-8 (1980); Mykoniatis, *J. Biochem. Biophys. Methods*, 10(5-6):321-8 (1985); Nagasawa et al., *J. Appl. Biochem.*, 7(4-5):296-302 (1985); Nikiforov and Rogers, *Anal. Biochem.*, 227(1):201-9 (1995); Proudnikov et al., *Anal. Biochem.*, 259(1):34-41 (1998); Rasmussen et al., *Anal. Biochem.*, 198(1):138-42 (1991); and

Rogers et al., *Anal. Biochem.*, 266(1):23-30 (1999).

Bresser et al., *DNA*, 2(3):243-54 (1983) discloses a method for selectively immobilizing either mRNA or DNA on nitrocellulose. Essential elements of the procedure for immobilizing DNA include tissue lysis, 5 proteinase K treatment, solubilization of nucleic acids in hot 12.2 molal NaI, passage through a nitrocellulose filter, and acetylation of residual protein with acetic anhydride. Advantages include speed, quantitative recovery, low background, and elimination of the usual baking step. Essential elements of the procedure for selectively immobilizing mRNA 10 include dissolving cells in Brij-35 and desoxycholate, proteinase K treatment, solubilizing nucleic acids in room temperature 12.2 molal NaI, filtration through nitrocellulose, and acetylation of residual protein. Advantages include selective immobilization of mRNA but not tRNA, rRNA, or DNA, and the maintenance of biological activity of the 15 immobilized mRNA.

Hirayama et al., *Nucleic Acids Res.*, 24(20):4098-9 (1996) discloses an improved and simplified protocol for DNA immobilization to enhance DNA-DNA hybridization on microwell plates. Target DNA was immobilized by simple dry-adsorption. Efficiencies of DNA immobilization 20 and retention were enhanced 1.4-6.5 times and 4.2-19.6 times, respectively, compared with a conventional method. The overall hybridization efficiency was increased 3.1-5.2 times. This simple new protocol can reduce the consumption of scarce DNA samples.

Kremsky et al., *Nucleic Acids Res.*, 15(7):2891-909 (1987) 25 discloses a general method for the immobilization of DNA through its 5'-end has. A synthetic oligonucleotide, modified at its 5'-end with an aldehyde or carboxylic acid, was attached to latex microspheres containing hydrazide residues. Using T4 polynucleotide ligase and an oligonucleotide splint, a single stranded 98mer was efficiently joined to 30 the immobilized synthetic fragment. After impregnation of the latex microspheres with the fluorescent dye, Nile Red and attachment of an

aldehyde 16mer,  $5 \times 10^5$  bead-DNA conjugates could be detected with a conventional fluorimeter.

Macdougall et al., *Biochem. J.*, 191(3):855-8 (1980) discloses a method in which double-stranded DNA is alkylated with

5 4-bis-(2-chloroethyl)amino-L-phenylalanine and the product immobilized on an insoluble support via the primary amino group of the phenylalanine moiety. The DNA is irreversibly bound to the matrix by both strands at a limited number of points.

Mykoniatis, *J. Biochem. Biophys. Methods*, 10(5-6):321-8 (1985)

10 discloses a method for the immobilization of DNA on Sephadex G200 in the presence of water soluble carbodiimide. An increase in the extent of binding was observed when the incubation temperature of the DNA-Sephadex mixture was changed. It was found that native DNA immobilized to Sephadex with higher efficiency than denatured DNA. The -  
15 stability of native DNA-Sephadex complex was about the same as that of denatured DNA-Sephadex. The size of DNA released by DNA-Sephadex after incubation of a suspension of the complex was the same as that of the DNA used for immobilization.

Nagasawa et al., *J. Appl. Biochem.*, 7(4-5):296-302 (1985)

20 discloses a method in which DNA was immobilized covalently to Sepharose by several methods using epichlorohydrin, cyanogen bromide, carbodiimide, hydroxysuccinimide, carbonyldiimidazole, trichlorotriazine, and diazonium salt. These immobilizing methods were compared from the standpoint of the preparation of immunosorbent for anti-DNA  
25 antibodies. Among these methods, that involving epichlorohydrin was the most suitable because of large coupling capacity, stability of bound DNA, and nonadsorption of anti-DNA by the support itself.

Nikiforov and Rogers, *Anal. Biochem.*, 227(1):201-9 (1995)

discusses 3 methods for the immobilization of relatively short (12-30 mer)  
30 oligonucleotide probes to 96-well polystyrene plates for use in DNA hybridization-based assays. Two of the methods are modifications of

previously published procedures, requiring the use of modified oligonucleotides and/or modified plates. These were compared to a newly developed method, whereby passive immobilization occurs by incubation in the presence of salt or a cationic detergent. While all 5 methods resulted in the productive binding of the DNA probes and could therefore be used for hybridization, only the passive immobilization approach met strict performance criteria for use in DNA genotyping.

Proudnikov et al., *Anal. Biochem.*, 259(1):34-41 (1998) discloses immobilization of DNA in polyacrylamide gel for the manufacture of DNA 10 and DNA-oligonucleotide microchips. Activated DNA was immobilized in aldehyde-containing polyacrylamide gel for use in manufacturing the MAGIChip (microarrays of gel-immobilized compounds on a chip). First, abasic sites were generated in DNA by partial acidic depurination. Amino groups were then introduced into the abasic sites by reaction with 15 ethylenediamine and reduction of the aldimine bonds formed. It was found that DNA could be fragmented at the site of amino group incorporation or preserved mostly unfragmented. In similar reactions, amino-DNA and amino-oligonucleotides were attached through their amines to polyacrylamide gel derivatized with aldehyde groups. Single- 20 and double-stranded DNA of 40 to 972 nucleotides or base pairs were immobilized on the gel pads to manufacture a DNA microchip. The microchip was hybridized with fluorescently labeled DNA-specific oligonucleotide probes. This procedure for immobilization of amino compounds was used to manufacture MAGIChips containing DNA and 25 oligonucleotides.

Rasmussen et al., *Anal. Biochem.*, 198(1):138-42 (1991) discloses covalent immobilization of DNA onto polystyrene microwells via the DNA's 5' end. DNA is bound onto the microwells by formation of a phosphoramidate bond between the 5' terminal phosphate group and the 30 microwells. Immobilization of 25 to 30 ng DNA per well is obtained. DNA molecules bound covalently at only the 5' end are, ideally, perfect

for hybridization.

Rogers et al., *Anal. Biochem.*, 266(1):23-30 (1999) discloses immobilization of oligonucleotides onto a glass support via disulfide bonds in preparation of DNA microarrays. This method provides an efficient and 5 specific covalent attachment chemistry for immobilization of DNA probes onto a solid support. Glass slides were derivatized with 3-mercaptopropyl silane for attachment of 5-prime disulfide-modified oligonucleotides via disulfide bonds. An attachment density of approximately  $3 \times 10^5$  oligonucleotides/micron<sup>2</sup> was observed. Oligonucleotides attached by this 10 method provided a highly efficient substrate for nucleic acid hybridization and primer extension assays. In addition, patterning of multiple DNA probes on a glass surface utilizing this attachment chemistry has been demonstrated, which allows for array densities of at least 20,000 spots/cm<sup>2</sup>.

15. I. HIGH-THROUGHPUT ASSAY FORMAT

Although the methods for detecting abnormal base-pairing, mutations or polymorphisms, or methods for removing or localizing such abnormal base-pairing described in Sections B-F can be used wherein a single sample is assayed in one assay, the assay is preferably conducted 20 in a high throughput mode, i.e., a plurality of the abnormal base-pairing, mutations or polymorphisms are detected, localized and/or removed simultaneously (See generally, *High Throughput Screening: The Discovery of Bioactive Substances* (Devlin, Ed.) Marcel Dekker, 1997; Sittampalam et al., *Curr. Opin. Chem. Biol.*, 1(3):384-91 (1997); and Silverman et al., 25 *Curr. Opin. Chem. Biol.*, 2(3):397-403 (1998)). For example, the assay can be conducted in a multi-well (e.g., 24-, 48-, 96-, or 384-well), chip or array format.

Current state-of-the-art high-throughput assay operations are highly automated and computerized to handle sample preparation, assay 30 procedures and the subsequent processing of large volumes of data. Each one of these steps requires careful optimization to operate

efficiently and can assay 100-300,000 samples in a 2-6 month period. Hence, a modern high-throughput assay operation is a multidisciplinary field involving analytical chemistry, biology, biochemistry, synthesis chemistry, molecular biology, automation engineering and computer science (Fernandes, *J. Biomol. Screening*, 2:1 (1997)).

1. High-throughput assay instrumentation and capabilities

In general, the instrumentation used in high-throughput assays should be accurate, reliable and easily amenable to automation.

Analytical methods should be robust and reproducible, with stable

10 reagents and signal responses. Signal-to-noise (S/N) ratios should be large enough to generate signal windows (Sittampalam et al., *J. Biomol. Screening*, 2:159-169 (1997)) that allow reliable detection of "hits".

2. Detection technologies

Detection technologies employed in high-throughput screens

15 depend on the type of biochemical pathway being investigated (Sittampalam et al., *Curr. Opin. Chem. Biol.*, 1(3):384-91 (1997)).

a. Radiochemical methods

Although filtration-based receptor binding assays have been used extensively in the past (to separate the bound and free radiolabeled

20 ligand), the scintillation proximity assay (SPA) has become the standard assay in many HTS operations, mainly because it does not require a separation step, and can be easily automated (Braunwalder et al., *J. Biomol. Screening*, 1:23-26 (1996); Cole, *Methods Enzymol.*, 275:310-328 (1996); Cook, *Drug Discov. Tech.*, 1:287-294 (1996); Kahl et al., *J.*

25 *Biomol. Screening*, 2:33-40 (1997); Lerner et al., *J. Biomol. Screening*, 1:135-143 (1996); Baker et al., *Anal. Biochem.*, 239:20-24 (1996); Baum et al., *Anal. Biochem.*, 237:129-134 (1996); Sullivan et al., *J. Biomol. Screening*, 2:19-23 (1997); De Serres et al., *Anal. Biochem.*, 233:228-233 (1996); Sonatore et al., *Anal. Biochem.*, 240:289-297

30 Chen et al., *J. Biol. Chem.*, 271:25308-25315 (1996); Patel et al., *Biochem. Biophys. Res. Commun.*, 221:821-825 (1996); and Fox,

*Pharm. Forum*, 6:1-3 (1996)). SPA can also be easily adapted to a variety of enzyme assays (Lerner et al., *J. Biomol. Screening*, 1:135-143 (1996); Baker et al., *Anal. Biochem.*, 239:20-24 (1996); Baum et al., *Anal. Biochem.*, 237:129-134 (1996); and Sullivan et al., *J. Biomol. Screening*, 2:19-23 (1997)) and protein-protein interaction assays (Braunwalder et al., *J. Biomol. Screening*, 1:23-26 (1996); Sonatore et al., *Anal. Biochem.*, 240:289-297 (1996); and Chen et al., *J. Biol. Chem.*, 271:25308-25315 (1996)).

One version of SPA utilizes polyvinyltoluene (PVT) microspheres or beads ( $\sim 5 \mu\text{m}$  diameter, density  $\sim 1.05 \text{ g/cm}^3$ ) into which a scintillant has been incorporated (Hook, *Drug Discov. Tech.*, 1:287-294 (1996)). When a radiolabeled ligand is captured on the surface of the bead, the radioactive decay occurs in close proximity to the bead, and effectively transfers energy to the scintillant, which results in light emission. When the radiolabel is displaced or inhibited from binding to the bead, it remains free in solution and is too distant from the scintillant for efficient energy transfer. Energy from radioactive decay is dissipated into the solution, which results in no light emission from the beads. Hence, the bound and free radiolabel can be detected without the physical separation required in filtration assays.

The ideal isotopes for labeling ligands used in SPA assays are  $^3\text{H}$  and  $^{125}\text{I}$ . This is because the  $\beta$  particles from  $^3\text{H}$  have a relatively short pathlength, about  $1.5 \mu\text{M}$ , which easily fulfills the distance requirement for SPA. The Auger electrons emitted by  $^{125}\text{I}$ , which travel between approximately  $1\mu\text{m}$  and  $17.6\mu\text{m}$  in aqueous media, also satisfy this distance requirement.

SPA can also be carried out in scintillating microplates (Braunwalder et al., *J. Biomol. Screening*, 1:23-26 (1996); Fox, *Pharm. Forum*, 6:1-3 (1996); and Harris et al., *Anal. Biochem.*, 243:249-256 (1996)), in which the scintillant is directly incorporated into the plastic, or is coated on the inner surface of the wells. These plates are

commercially available. For example, Flashplate® is from NEN™ Life Science Products (Boston, MA) in which the scintillant is coated on the inner surface of the wells. The Scinitstrip® plate is from WallacOy (Turku, Finland) which is made by incorporating the scintillant into the entire plastic. A more recent development is the Cytostar-T™ (Amerisham Life Sciences, Cardiff, Wales) scintillating microplates (Fox, *Pharm. Forum*, 6:1-3 (1996) which were specially designed for cell-based proximity assays. Scintillant is incorporated into the base plate of microtiter plates and can also detect additional isotopes such as <sup>14</sup>C, <sup>45</sup>Ca, <sup>35</sup>S, and <sup>33</sup>P.

**b. Non-isotopic detection methods**

**1) Colorimetry and luminescence**

Colorimetric and luminescence detection methods have significant advantages for HTS laboratories, particularly in light of the cost, safety and disposal issues associates with radiochemical methods. Since luminescence methods can be as sensitive as radioactive methods, with low detection limits, these techniques are being used increasingly in HTS assays (Brown et al., *Curr. Opin. Biotechnol.*, 8:45-49 (1997); Glazer, *BioRadiations*, 98:4-8 (1997); Czarnik, *Chem. Biol.*, 2:423-428 (1995); Wang et al., *Tetrahedron Lett.*, 31:6493-6496 (1991); Mathis, *Clin. Chem.*, 41:1391-1397 (1995); Kolb et al., *J. Biomol. Screening*, 1:203-210 (1996); Gonzalez et al., *Biophys. J.*, 69:1272-1280 (1995); Schroeder et al., *J. Biomol. Screening*, 1:75-80 (1996); Waggoner et al., *Hum. Pathol.*, 27:494-502 (1996); Jameson et al., *Methods Enzymol.*, 246:283-300 (1995); Lundblad et al., *Mol. Endocrinol.*, 10:607-612 (1996); Checovich et al., *Nature*, 375:254-256 (1995); Levine et al., *Anal. Biochem.*, 247:83-88 (1997); Jolley, *J. Biomol. Screening*, 1:33-38 (1996); Schade et al., *Anal. Biochem.*, 243:1-7 (1996); Lynch et al., *Anal. Biochem.*, 247:77-82 (1997); Sterrer et al., *J. Recept. Signal Transduct Res.*, 17:511-520 (1997); Rigler, *J. Biotechnol.*, 41:177-186 (1995); Rauer et al., *Biophys. Chem.*, 58:3-12 (1996); Sarubbi et al.,

*Anal. Biochem.*, 237:70-75 (1996); Rose et al., *Network Science*, 2(9):1-12 (1996); Dhundale et al., *J. Biomol. Screening*, 1:115-118 (1996); Suto et al., *J. Biomol. Screening*, 2:7-9 (1997); Bronstein et al., *Anal. Biochem.*, 219:169-181 (1994); Hastings, *Gene*, 173:5-11 (1996); Lehel et al., *Anal. Biochem.*, 244:340-346 (1997); Kolb et al., *J. Biomol. Screening*, 1:85-88 (1996); Bran et al., *J. Biomol. Screening*, 1:43-45 (1996); Rizzuto et al., *Curr. Biol.*, 6:183-188 (1996)). Glazer (Glazer, *BioRadiations*, 98:4-8 (1997)) and Czarnik (Czarnik, *Chem. Biol.*, 2:423-428 (1995)) and the Fluorescent Chemosensors and Biosensors Database 10 on the World Wide Web URL; <http://biomednet.com/fluoro/> have reviewed the utility and need for fluorescence-based techniques for biological applications, which can be easily extended to HTS assays.

### 3) Resonance energy transfer

Resonance energy transfer (RET) between a fluorophore and chromophore was one of the earliest methods developed for HTS. For example, a peptide substrate for an HIV protease was synthesized with EDANS (as the amino terminus) as the donor fluorophore, and DABCYL (at the carboxyl terminus) as the acceptor chromophore (Wang et al., *Tetrahedron Lett.*, 31:6493-6496 (1991)). Energy transfer from EDANS 15 to DABCYL in the intact peptide resulted in quenching of EDANS fluorescence.

### 3) Time-resolved fluorescence

A new homogeneous time-resolved fluorescence (HTRF) technology has been described (Mathis, *Clin. Chem.*, 41:1391-1397 25 (1995)). The assay utilizes fluorescence energy transfer between two fluorophores (an europium cryptate and a 105kDa phycobiliprotein, allophycocyanin) as labels. The Eu-trisbipyridine cryptate (TBP-EU<sup>3+</sup>,  $\lambda_{ex}$  = 337 nm) has two bipyridyl groups that harvest light and channel it to the caged Eu<sup>3+</sup>. It has a long fluorescence, lifetime and nonradioactively 30 transfers the energy to allophycocyanin when the two labels are in close proximity (>50% transfer efficiency at a donor-acceptor distance of 9.5

nm). The resulting fluorescence of allophycocyanin ( $\lambda_{em} = 665$  nm) retains the long lifetime of the donor TBP-EU<sup>3+</sup>, allowing time-resolved measurement. These labels and their spectroscopic characteristics are very stable in biological media.

5                  4) Cell-based fluorescence assays

An interesting fluorescence resonance energy transfer (FRET) procedure for sensing voltage across cell membranes has been described recently (Gonzalez et al., *Biophys. J.*, 69:1272-1280 (1995)). The technique uses membrane permeable, anionic oxonols which rapidly 10 locate on the inner or outer membrane surface depending on polarization state of the membrane. FRET occurs between fluorescein-labeled WGA and the oxonols bound to the other surface of the membrane at a resting negative potential. As a positive potential, the oxonols are relocated to the inner membrane surface, and the FRET is greatly reduced.

15        Many fluorescence intensity measurements, including FRET, can be configured on instruments specifically designed for cell-based HTS assays in 96-well or higher density plates called FLIPR (Schroeder et al., *J. Biomol. Screening*, 1:75-80 (1996)). FLIPR utilizes a water-cooled argon ion laser (5 watt) or a xenon arc lamp and a confocal optical 20 system with a charge-coupled device (CCD) camera to illuminate and image the entire plate. The spatial resolution of the optics is ~200  $\mu\text{m}$  at the cell plane. The plate chamber temperature can be controlled precisely, and a 96-well pipettor head is integrated into the instrument. These features allow accurate measurements of cellular biochemistry in 25 confluent layers of cells at the bottom of plates. FLIPR software can rapidly quantify transient fluorescence signals in intact cells that are growing attached to the bottom of the well. HTS assays involving intracellular calcium, pH and membrane potential measurements have been designed using this instrument (Waggoner et al., *Hum. Pathol.*, 30 27:494-502 (1996)).

### 5) Fluorescence polarization

Another technique that has gained popularity recently is fluorescence polarization or anisotropy (Jameson et al., *Methods Enzymol.*, 246:283-300 (1995); Lundblad et al., *Mol. Endocrinol.*, 10:607-612 (1996); Checovich et al., *Nature*, 375:254-256 (1995); Levine et al., *Anal. Biochem.*, 247:83-88 (1997); Jolley, *J. Biomol. Screening*, 1:33-38 (1996); Schade et al., *Anal. Biochem.*, 243:1-7 (1996); Lynch et al., *Anal. Biochem.*, 247:77-82 (1997)). When fluorescently labeled molecules in solution are illuminated with plane-polarized light, the emitted fluorescence will be in the same plane provided the molecules remain stationary. Since all molecules tumble as a result of collisional motion, depolarization phenomenon is proportional to the rotational relaxation time ( $\mu$ ) of the molecule, which is defined by the expression  $3\eta V/RT$ . At constant viscosity ( $\eta$ ) and temperature (T) of the solution, polarization is directly proportional to the molecular volume (V) (R is the universal gas constant). Hence, changes in molecular volume or molecular weight due to binding interactions can be detected as a change in polarization. For example, the binding of a fluorescently labeled ligand to its receptor will result in significant changes in measured fluorescence polarization values for the ligand. Once again, the measurements can be made in a "mix and measure" mode without physical separation of the bound and free ligands. The polarization measurements are relatively insensitive to fluctuations in fluorescence intensity when working in solutions with moderate optical intensity.

### 25 6) Fluorescence correlation spectroscopy

Fluorescence correlation spectroscopy (FCS) has been recently described for HTS applications (Sterrer et al., *J. Recept. Signal Transduct Res.*, 17:511-520 (1997); Rigler, *J. Biotechnol.*, 41:177-186 (1995); Rauer et al., *Biophys. Chem.*, 58:3-12 (1996)). FCS measures time-dependent and spontaneous fluctuations in fluorescence intensities in very small volumes (nanoliters). These fluctuations usually result from

Brownian motion associated with chemical reactions, diffusion or the flow of fluorescently labeled molecules. The average fluctuation is proportional to the square root of N, where N is the average number of molecules in the volume. Since Brownian diffusion is directly affected by 5 molecular interactions, FCS is an excellent tool to measure binding interactions (Brown et al., *Curr. Opin. Biotechnol.*, 8:45-49 (1997)). Using powerful lasers and autocorrelation techniques, sensitive measurements (at concentrations of ~ $10^{-12}$ M) can be made in solution and in cellular compartments.

10        3.     Miniaturization

Several factors are fueling efforts to increase the speed of HTS and decrease the volume of individual reactions within an HTS format (Silverman et al., *Curr. Opin. Chem. Biol.*, 2(3):397-403 (1998)). Split-bead synthesis, or other similar approaches to combinatorial chemistry, 15 dramatically increases the number of compounds that can be produced in a library but do so at the cost of quantity of material.

One approach involves reducing the well size and increasing the density of the assay plate but retaining the overall assay format used in current 96-well based HTS. Densities of 6,500 assays in a 10 cm array 20 have been reported to cell-free enzyme based assays (Schullek et al., *Anal. Biochem.*, 246:20-29 (1997)) and for ligand binding in cell based assays (You et al., *Chem. Biol.*, 4:969-975 (1997)). This approach of miniaturizing existing formats significantly increases the number of assays per plate and the overall throughput of the screen but is intrinsically 25 limited by the physical constraints of delivering small volumes to wells, and of detecting responses in a sensitive and timely manner. Another approach uses glass chips containing microchannels in which reagents, target proteins and compounds are herded by electrokinetic flow controlled by electric potentials applied at the ends of the channels (Hadd 30 et al., *Anal. Chem.*, 69:3407-3412 (1997)). A related approach attains high-throughput of chemical synthesis and activity assessment by parallel

arrays of three-dimensional channels in which flow is controlled by miniature hydrostatic actuators (Rogers, *Drug Discov. Today*, 2:306 (1997)). These approaches provide significant reduction in the volume of assays and a corresponding savings in reagent costs over conventional

5 HTS. In addition, with further development in parallel processing in multiple chips, the number of assays performed in a given period of time can increase dramatically.

In a specific embodiment, the HTS methods disclosed in the following literatures can be used, with or without modification, in the  
10 present methods for detecting, localizing and/or removing abnormal base-pairing, mutations and polymorphisms: Janzen et al., The 384-well plate: pros and cons, *J. Biomol. Screening*, 1:63-64 (1996); Lutz, et al., Experimental design for high-throughput screening, *Drug Discov. Tech.*, 1:277-286 (1996); Klein, et al., Recombinant microorganisms as tools for  
15 high throughput screening for non antibiotic compounds, *J. Biomol. Screening*, 2:41-49 (1997); Webb, et al., Transcription-specific assay for quantifying mRNA: A potential replacement for reporter gene assays, *J. Biomol. Screening*, 1:119-121 (1996); Charych, et al., Direct colorimetric detection of receptor-ligand interaction by a polymerized bilayer assembly,  
20 *Science*, 261:585-588 (1993); Charych, et al., A 'litmus test' for molecular recognition using artificial membranes, *Chem. Biol.*, 3:113-120 (1996); Spevak, et al., Carbohydrates in an acidic multivalent assembly: nanomolar P-selectin inhibitors, *J. Med. Chem.*, 38:1018-1020 (1996);  
Allen, et al., Atomic force microscopy in analytical biotechnology, *Trends Biotechnol.*, 15:101-105 (1997); Troy, et al., Scanning force microscopy helps in the design of cancer drugs, *Biophoton Int.*, 9/10:52-53 (1996);  
Paborsky, et al., A nickel chelate microtiter plate assay for six histidine-containing proteins, *Anal. Biochem.*, 234:60-65 (1996); Weiss-Wichert, et al., A new analytical device based on gated ion channels: A peptide  
25 channel biosensor, *J. Biomol. Screening*, 2:11-18 (1997); Brecht, et al., Transducer-based approaches for parallel binding assays in HTS, *J.*

*Biomol. Screening*, 1:191-201 (1996); Tyagi, et al., Molecular beacons: probes that fluoresce upon hybridization, *Nat. Biotechnol.*, 14:303-308 (1996); Heller, et al., Discovery and analysis of inflammatory disease-related genes using cDNA microarrays, *Proc. Natl. Acad. Sci. USA*, 94:2150-2155 (1997); Nicolaou, et al., Radiofrequency encoded combinatorial chemistry, *Angew Chem. Int. Ed.*, 34:2289-2291 (1995); Fitzgerald, et al., Direct characterization of solid phase resin-bound molecules by mass spectrometry, *Bioorg. Med. Chem. Lett.*, 6:979-982 (1996); Chu, et al., Affinity capillary electrophoresis-mass spectrometry for screening combinatorial libraries, *J. Am. Chem. Soc.*, 118:7827-7835 (1996); and Evans, et al., Affinity-based screening of combinatorial libraries using automated, serial-column chromatography, *Nat. Biotechnol.*, 14:504-507 (1996).

#### J. SAMPLE COLLECTION

Any sample can be assayed for detecting, localizing and/or removing abnormal base-pairing, mutations or polymorphisms using the methods described in the above Sections B-F. In one embodiment, the sample being assayed is a biological sample from a mammal, particularly a human, such as a biological fluid or a biological tissue. Biological fluids, include, but are not limited to, urine, blood, plasma, serum, saliva, semen, stool, sputum, hair and other keratinous samples, cerebral spinal fluid, tears, mucus and amniotic fluid. Biological tissues contemplated include, but are not limited to, aggregates of cells, usually of a particular kind together with their intercellular substance that form one of the structural materials of a human, animal, plant, bacterial, fungal or viral structure, including connective, epithelium, muscle and nerve tissues, organs, tumors, lymph nodes, arteries and individual cell(s). In one specific embodiment, the body fluid to be assayed is urine. In another specific embodiment, the body fluid to be assayed is blood. Preferably, the blood sample is further separated into a plasma or sera fraction.

Serum or plasma can be recovered from the collected blood by any

methods known in the art. In one specific embodiment, the serum or plasma is recovered from the collected blood by centrifugation. Preferably, the centrifugation is conducted in the presence of a sealant having a specific gravity greater than that of the serum or plasma and

5 less than that of the blood corpuscles which will form the lower, whereby upon centrifugation, the sealant forms a separator between the upper serum or plasma layer and the lower blood corpuscle layer. The sealants that can be used in the processes include, but are not limited to, styrene resin powders (Japanese Patent Publication No. 38841/1973), pellets or

10 plates of a hydrogel of a crosslinked polymer of 2-hydroxyethyl methacrylate or acrylamide (U.S. Patent No. 3,647,070), beads of polystyrene bearing an antithrombus agent or a wetting agent on the surfaces (U.S. Patent No. 3,464,890) and a silicone fluid (U.S. Patent Nos. 3,852,194 and 3,780,935). In a preferred embodiment, the sealant

15 is a polymer of unsubstituted alkyl acrylates and/or unsubstituted alkyl methacrylates, the alkyl moiety having not more than 18 carbon atoms, the polymer material having a specific gravity of about 1.03 to 1.08 and a viscosity of about 5,000 to 1,000,000 cps at a shearing speed of about 1 second<sup>-1</sup> when measured at about 25°C (U.S. Patent No.

20 4,140,631).

In another specific embodiment, the serum or plasma is recovered from the collected blood by filtration. Preferably, the blood is filtered through a layer of glass fibers with an average diameter of about 0.2 to 5  $\mu$  and a density of about 0.1 to 0.5 g./cm<sup>3</sup>, the total volume of the

25 plasma or serum to be separated being at most about 50% of the absorption volume of the glass fiber layer; and collecting the run-through from the glass fiber layer which is plasma or serum (U.S. Patent No. 4,477,575). Also preferably, the blood is filtered through a layer of glass fibers having an average diameter 0.5 to 2.5  $\mu$  impregnated with a

30 polyacrylic ester derivative and polyethylene glycol (U.S. Patent No. 5,364,533). More preferably, the polyacrylic ester derivative is

poly(butyl acrylate), poly(methyl acrylate) or poly(ethyl acrylate), and (a) poly(butyl acrylate), (b) poly(methyl acrylate) or poly(ethyl acrylate) and (c) polyethylene glycol are used in admixture at a ratio of (10-12):(1-4):(1-4). In still another specific embodiment, the serum or plasma is recovered from the collected blood by treating the blood with a coagulant containing a lignan skelton having oxygen-containing side chains or rings (U.S. Patent No. 4,803,153). Preferably, the coagulant contains a lignan skelton having oxygen-containing side chains or rings, e.g., d-sesamin, l-sesamin, paulownin, d-asarinin, l-asarinin, 2 $\alpha$ -paulownin, 6 $\alpha$ -paulownin, 10 pinoresinol, d-eudesmin, l-pinoresinol  $\beta$ -D-glucoside, l-pinoresinol, l-pinoresinol monomethyl ether  $\beta$ -D-glucoside, epimagnolin, lirioresinol-B, syringaresinol (dl), lirioresinonB-dimethyl ether, phillyrin, magnolin, lirioresinol-A, 2 $\alpha$ , 6 $\alpha$ -d-sesamin, d-diaeudesmin, lirioresinol-C dimethyl ether (d-diayangambin) and sesamolin. More preferably, the coagulant is used in an amount ranging from about 0.01 to 50 g per 1 l of the blood.

#### K. COMBINATIONS, KITS AND ARTICLES OF MANUFACTURE

Combinations, kits and articles of manufacture for detecting abnormal base-pairings, mutations, polymorphisms, and for localizing and/or removing abnormal base-pairings are provided herein.

20 In a specific embodiment, a combination for detecting abnormal base-pairing in a nucleic acid duplex is provided herein, which combination comprises: a) a mutant DNA repair enzyme or complex thereof; and b) reagents for detecting binding between abnormal base-pairing in a nucleic acid duplex and the mutant DNA repair enzyme or complex thereof. A kit comprising the above combination is also provided. An article of manufacture is further provided herein, which article of manufacture comprises: a) packaging material; b) the above-described combination; and c) a label indicating that the article is for use in detecting abnormal base-pairing in a nucleic acid duplex.

25 30 In another specific embodiment, a combination for detecting a mutation in a nucleic acid duplex is provided herein, which combination

comprises: a) a strand of a wild-type nucleic acid complementary to a nucleic acid having or suspected of having a mutation; b) a mutant DNA repair enzyme or complex thereof; and c) reagents for detecting binding between abnormal base-pairing in a nucleic acid duplex and the mutant

5     DNA repair enzyme or complex thereof. A kit comprising the above combination is also provided. An article of manufacture is further provided, comprising: a) packaging material; b) the above combination; and c) a label indicating that the article is for use in detecting a mutation in a nucleic acid duplex.

10    In still another specific embodiment, a combination for detecting a polymorphism in a locus is provided herein, which combination comprises: a) a complementary reference strand of a nucleic acid comprising a known allele of a locus; b) a mutant DNA repair enzyme or complex thereof; and c) reagents for detecting binding between abnormal

15    base-pairing in a nucleic acid duplex and the mutant DNA repair enzyme or complex thereof. A kit comprising the above combination is also provided. An article of manufacture is further provided, comprising: a) packaging material; b) the above combination; and c) a label indicating that the article is for use in detecting a polymorphism in a locus.

20    In yet another specific embodiment, a combination for removing a nucleic acid duplex containing one or more abnormal base-pairing in a population of nucleic acid duplexes is provided herein, which combination comprises: a) a mutant DNA repair enzyme or complex thereof; and b) reagents for removing a binding complex formed between a nucleic acid

25    duplex containing one or more abnormal base-pairing and the mutant DNA repair enzyme or complex thereof. A kit comprising the above combination is also provided. An article of manufacture is further provided, comprising: a) packaging material; b) the above combination; and c) a label indicating that the article is for use in removing a nucleic

30    acid duplex containing one or more abnormal base-pairing in a population of nucleic acid duplexes.

In yet another specific embodiment, a combination for detecting and localizing an abnormal base-pairing in a nucleic acid duplex is provided herein, which combination comprises: a) a mutant DNA repair enzyme or complex thereof; and b) an exonuclease. A kit comprising the 5 above combination is also provided. An article of manufacture is further provided, comprising: a) packaging material; b) the above combination; and c) a label indicating that the article is for use in for detecting and localizing an abnormal base-pairing in a nucleic acid duplex.

Since modifications will be apparent to those of skill in this art, it is 10 intended that this invention be limited only by the scope of the appended claims.

**CLAIMS:**

1. A method for detecting abnormal base-pairing in a nucleic acid duplex, which method comprises:
  - a) contacting a nucleic acid duplex having or suspected of
  - 5 having an abnormal base-pairing with a mutant nucleic acid repair enzyme or complex thereof, wherein the mutant nucleic acid repair enzyme or complex thereof has binding affinity for the abnormal base-pairing in the duplex but has attenuated catalytic activity compared to the wild-type enzyme; and
- 10 b) detecting binding between the nucleic acid duplex and the mutant nucleic acid repair enzyme or complex thereof, whereby the presence or quantity of the abnormal base-pairing in the duplex is assessed.
2. The method of claim 1, wherein the nucleic acid duplex is selected from the group consisting of a DNA:DNA, a DNA:RNA and an RNA:RNA duplex.
- 15 3. The method of claim 2, wherein the nucleic acid duplex is a DNA:DNA duplex.
4. The method of claim 1, wherein the abnormal base-pairing is selected from the group consisting of a base-pair mismatch, a base insertion, a base deletion and a pyrimidine dimer.
- 20 5. The method of claim 4, wherein the base-pair mismatch is a single base-pair mismatch.
6. The method of claim 1, wherein the mutant nucleic acid repair enzyme or enzyme complex is selected from the group consisting of a mutant mutH, a mutant mutL, a mutant mutM, a mutant mutS, a mutant mutY, a mutant uvrD, a mutant dam, a mutant thymidine DNA glycosylase (TDG), a mutant mismatch-specific DNA glycosylase (MUG), a mutant AlkA, a mutant MLH1, a mutant MSH2, a mutant MSH3, a
- 25 30 mutant MSH6, a mutant Exonuclease I, a mutant T4 endonuclease V, a mutant FEN1 (RAD27), a mutant DNA polymerase  $\delta$ , a mutant DNA

polymerase ε, a mutant RPA, a mutant PCNA, a mutant RFC, a mutant Exonuclease V, a mutant DNA polymerase III holoenzyme, a mutant DNA helicase, a mutant RecJ exonuclease and combinations thereof.

7. The method of claim 1, wherein the nucleic acid duplex is  
5 formed by hybridizing a single strands of nucleic acid that contain a known sequence with a nucleic acids from a test sample, whereby binding of the mutant enzyme to any duplexes indicates that presence of a sequence difference in the nucleic acid from the sample from that of the nucleic acid containing the known sequence.

10 8. The method of claim 1, wherein the single strands of nucleic acid fragments with known sequences are immobilized on a solid support.

9. The method of claim 8, wherein the fragments are arranged in an array.

10. The method of claim 8 that is automated.

15 11. A method for detecting a mutation in a nucleic acid, comprising:

20 a) hybridizing a strand of a nucleic acid having or suspected of having a mutation with a complementary strand of a nucleic acid fragment having a wild type sequence, whereby the mutation results in an abnormal base-pairing in the formed nucleic acid duplex;

25 b) contacting the nucleic acid duplex formed in step a) with a mutant nucleic acid repair enzyme or complex thereof, wherein the mutant nucleic acid repair enzyme or complex thereof has binding affinity for the abnormal base-pairing in the duplex but has attenuated catalytic activity; and

c) detecting binding between the nucleic acid duplex and the mutant nucleic acid repair enzyme or complex thereof, whereby the presence or quantity of the mutation is assessed.

30 12. The method of claim 11, wherein the nucleic acid strand to be tested and the complementary wild-type nucleic acid strand are NA strands.

13. The method of claim 11, wherein the mutation is associated with a disease or disorder, or infection by a pathological agent, and the method is used for prognosis or diagnosis of the presence or severity of the disease, disorder or infection.

5 14. The method of claim 13, wherein the disease or disorder is selected from the group consisting of a cancer, an immune system disease or disorder, a metabolism disease or disorder, a muscle and bone disease or disorder, a nervous system disease or disorder, a signal disease or disorder and a transporter disease or disorder.

10 15. The method of claim 13, wherein a plurality of mutations are identified by hybridizing nucleic acid single strands to a plurality of different fragments comprising loci encompassing different mutations.

16. The method of claim 15 that is automated.

17. A method for detecting polymorphism in a gene locus,  
15 comprising:

a) hybridizing a target strand of a nucleic acid comprising a locus to be tested with a complementary reference strand of a nucleic acid comprising a known allele of the locus, whereby the allelic identity between the target and the reference strands results in the formation of a 20 nucleic acid duplex without an abnormal base-pairing and the allelic difference between the target and the reference strands results in the formation of a nucleic acid duplex with an abnormal base-pairing;

b) contacting the nucleic acid duplex formed in step a) with a mutant nucleic acid repair enzyme or complex thereof, wherein the 25 mutant nucleic acid repair enzyme or complex thereof has binding affinity for the abnormal base-pairing in the duplex but has attenuated catalytic activity; and

c) detecting binding between the nucleic acid duplex and the mutant nucleic acid repair enzyme or complex thereof, whereby the 30 polymorphism in the locus is assessed.

18. The method of claim 17, wherein a plurality of reference

strands are hybridized.

19. The method of claim 18, wherein the reference strands are immobilized on a solid support.

5 20. The method of claim 19, wherein the reference strands are immobilized in an array.

21. The method of claim 17, wherein the polymorphism to be detected is a variable nucleotide type polymorphism ("VNTR").

10 22. The method of claim 17, wherein the polymorphism to be detected is a single nucleotide polymorphism (SNP).

23. The method of claim 22, wherein the SNP is a human genome SNP.

15 24. The method of claim 23, wherein the hybridization between the target strand of a nucleic acid comprising a locus to be tested and the complementary reference strand of a nucleic acid comprising a known allele of the locus is facilitated by a recombinase.

25. The method of claim 18 that is automated.

20 26. A method for purifying or separating nucleic acid duplex containing one or more abnormal base-pairing from a population of nucleic acid duplexes, which method comprises:

25 a) contacting a population of nucleic acid duplexes having or suspected of having a nucleic acid duplex containing one or more abnormal base-pairing with a mutant nucleic acid repair enzyme or complex thereof, wherein the mutant nucleic acid repair enzyme or complex thereof has binding affinity for the abnormal base-pairing in the duplex but has attenuated catalytic activity and whereby the nucleic acid duplex containing one or more abnormal base-pairing binds to the mutant nucleic acid repair enzyme or complex thereof to form a binding complex; and

30 b) removing nucleic acid duplexes that contain the binding complex formed in step a) from the population of nucleic acid duplexes.

27. The method of claim 1, wherein the abnormal base-pairing is selected from the group consisting of a base-pair mismatch, a base insertion, a base deletion and a pyrimidine dimer.

28. The method of claim 11, wherein the abnormal base-pairing is selected from the group consisting of a base-pair mismatch, a base insertion, a base deletion and a pyrimidine dimer.

29. The method of claim 26, wherein the abnormal base-pairing is selected from the group consisting of a base-pair mismatch, a base insertion, a base deletion and a pyrimidine dimer.

10 30. The method of claim 26, wherein the population of nucleic acid duplexes is produced by an enzymatic amplification.

31. A method for detecting and localizing an abnormal base-pairing in a nucleic acid duplex, which method comprises:

15 a) contacting a nucleic acid duplex having or suspected of having an abnormal base-pairing with a mutant nucleic acid repair enzyme or complex thereof, wherein the mutant nucleic acid repair enzyme or complex thereof has binding affinity for the abnormal base-pairing in the duplex but has attenuated catalytic activity and whereby the nucleic acid duplex containing an abnormal base-pairing binds to the mutant nucleic acid repair enzyme or complex thereof to form a binding complex;

20 b) subjecting the nucleic acid duplex to hydrolysis with an exonuclease under conditions such that the binding complex formed in step a) blocks hydrolysis; and

25 c) determining the location within the nucleic acid duplex protected from the hydrolysis, thereby detecting and localizing the abnormal base-pairing in the nucleic acid duplex.

32. The method of claim 31, wherein the nucleic acid duplex is selected from the group consisting of a DNA:DNA, a DNA:RNA and a RNA:RNA duplex.

30 33. The method of claim 31, wherein the abnormal base-pairing is selected from the group consisting of a base-pair mismatch, a base

insertion, a base deletion and a pyrimidine dimer.

34. The method of claim 31, wherein the exonuclease is selected from the group consisting of nuclease BAL-31, exonuclease III, Mung Bean exonuclease and Lambda exonuclease.

5 35. The method of claim 1, wherein the mutant nucleic acid repair enzyme or complex thereof is labelled with a detectable label.

36. The method of claim 35, wherein the mutant nucleic acid repair enzyme or complex thereof is labelled with biotin.

10 37. The method of claim 36, wherein the binding between the abnormal base-pairing and the biotin-labelled mutant nucleic acid repair enzyme or complex thereof is detected with a streptavidin labeled enzyme.

15 38. The method of claim 37, wherein the streptavidin labeled enzyme is selected from the group consisting of a peroxidase, an urease, an alkaline phosphatase, a luciferase and a glutathione S-transferase.

39. The method of claim 31, wherein the mutant nucleic acid repair enzyme or complex thereof is labelled.

40. The method of claim 11, wherein the mutant nucleic acid repair enzyme or complex thereof is labelled with a detectable label.

20 41. The method of claim 17, wherein the mutant nucleic acid repair enzyme or complex thereof is labelled with a detectable label.

42. The method of claim 26, wherein the mutant nucleic acid repair enzyme or complex thereof is labelled with a detectable label.

25 43. The method of claim 1, wherein the nucleic acid duplex or the mutant nucleic acid repair enzyme or complex thereof is immobilized on the surface of a support.

44. The method of claim 43, wherein the nucleic acid duplex or the mutant nucleic acid repair enzyme or complex thereof is immobilized directly on the surface or is immobilized on the surface via a linker.

30 45. The method of claim 43, wherein the insoluble support is a silicon chip.

46. The method of claim 45, wherein the geometry of the support is selected from the group consisting of beads, pellets, disks, capillaries, hollow fibers, needles, solid fibers, random shapes, thin films, membranes and chips.

5 47. The method of claim 44, wherein the nucleic acid duplex or the mutant nucleic acid repair enzyme or complex thereof is immobilized in an array or a well format on the surface.

10 48. The method of claim 11, wherein the strand of a nucleic acid having or suspected of having a mutation, the complementary strand of a wild-type nucleic acid, or the mutant nucleic acid repair enzyme or complex thereof is immobilized on the surface of a support.

15 49. The method of claim 17, wherein the target strand of a nucleic acid comprising a locus to be tested, the complementary reference strand of a nucleic acid comprising a known allele of the locus , or the mutant nucleic acid repair enzyme or complex thereof is immobilized on the surface of a support.

50. The method of claim 26, wherein the mutant nucleic acid repair enzyme or complex thereof is immobilized on the surface of a support.

20 51. The method of claim 31, wherein the nucleic acid duplex having or suspected of having an abnormal base-pairing or the mutant nucleic acid repair enzyme or complex thereof is immobilized on the surface of a support.

25 52. The method of claim 1, wherein the nucleic acid duplex having or suspected of having an abnormal base-pairing is isolated from a sample.

53. The method of claim 52, wherein the sample is a body fluid or a biological tissue.

30 54. The method of claim 53, wherein the body fluid is selected from the group consisting of urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, mucus and amniotic fluid.

55. The method of claim 53, wherein the biological tissue is selected from the group consisting of connective tissue, epithelium tissue, muscle tissue, nerve tissue, organs, tumors, lymph nodes, arteries and individual cell(s).

5 56. The method of claim 11, wherein the strand of a nucleic acid having or suspected of having a mutation is isolated from a sample.

57. The method of claim 17, wherein the strand of a nucleic acid comprising a locus to be tested is isolated from a sample.

58. The method of claim 26, wherein the population of nucleic acid duplexes is isolated from a sample.

59. The method of claim 31, wherein the nucleic acid duplex having or suspected of having an abnormal base-pairing is isolated from a sample.

60. The method of claim 1, wherein abnormal base-pairings in a plurality of the nucleic acid duplexes are detected simultaneously.

61. The method of claim 11, wherein mutations in a plurality of the nucleic acids are detected simultaneously.

62. The method of claim 17, wherein polymorphisms in a plurality of the loci are detected simultaneously.

63. The method of claim 26, wherein a plurality of nucleic acid duplexes containing one or more abnormal base-pairing are removed simultaneously.

64. The method of claim 31, wherein a plurality of the abnormal base-pairings are detected and localized simultaneously.

65. A combination for detecting abnormal base-pairing in a nucleic acid duplex, which combination comprises:

a) a mutant nucleic acid repair enzyme or complex thereof; and  
b) a reagent for detecting binding between abnormal base-pairing in a nucleic acid duplex and the mutant nucleic acid repair enzyme

30 or complex thereof.

66. A kit comprising the combination of claim 65 and

instructions for binding the mutant repair enzyme to nucleic acid duplexes to detect a mutation in a nucleic acid duplex, or to detect a polymorphism in a locus, or diagnose a disease or disorder or plurality thereof, or for gene mapping or identification by detecting a plurality of polymorphisms  
5 or mutations.

67. An isolated substantially pure mutant nucleic acid repair enzyme that further comprises a detectable label, wherein the mutant enzyme has attenuated catalytic activity compared to the wild type but retains binding affinity for a nucleic acid duplex containing an abnormal  
10 base pairing.

68. The mutant enzyme of claim 67 that comprises a fusion protein or conjugate of the mutant enzyme and an enzyme label.

69. An isolated substantially pure biotinylated mutant nucleic acid repair enzyme.

15 70. An article of manufacture, comprising:

a) packaging material;  
b) a mutant nucleic acid repair enzyme that has attenuated catalytic activity compared to the wild type but retains binding affinity for a nucleic acid duplex containing an abnormal base pairing; and

20 c) a label indicating that the article is for use in detecting abnormal base-pairing in a nucleic acid duplex.

71. A combination for detecting and localizing an abnormal base-pairing in a nucleic acid duplex, comprising

a) a mutant nucleic acid repair enzyme or complex thereof,  
25 wherein the mutant enzyme that has attenuated catalytic activity compared to the wild type but retains binding affinity for a nucleic acid duplex containing an abnormal base pairing; and  
b) an exonuclease.

72. A kit, comprising the combination of claim 71 and  
30 instructions for performing an assay for detecting and localizing an abnormal base-pairing in a nucleic acid duplex.

1  
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Pro Val Pro Arg Pro Ala Ser Arg Ser Ser Pro Gly Ala Lys Arg  
65 70 75 80  
Arg His Ser Cys Ala Glu Ala Leu Val Ala Pro Leu Pro Ala Ala Ser  
85 90 95  
Pro Gln Arg Ser Arg Ser Pro Ser Pro Gln Pro Ser Pro His Val Ala  
100 105 110  
Pro Gln Asp Asp Ser Ile Pro Ala Gly Tyr Pro Pro Thr Ala Gly Ser  
115 120 125  
Ala Val Leu Met Asp Ala Leu Asn Thr Leu Ala Thr Asp Ser Pro Cys  
130 135 140  
Gly Ile Pro Ser Lys Ile Trp Lys Thr Ser Pro Asp Pro Thr Pro Val  
145 150 155 160  
Ser Thr Ala Pro Ser Lys Ala Gly Leu Ala Arg His Ile Tyr Pro Thr  
165 170 175  
Val Glu Phe Leu Gly Pro Cys Glu Gln Glu Glu Arg Arg Asn Ser Ala  
180 185 190  
Pro Glu Ser Ile Leu Leu Val Pro Pro Thr Trp Pro Lys Gln Leu Val  
195 200 205  
Pro Ala Ile Pro Ile Cys Ser Ile Pro Val Thr Ala Ser Leu Pro Pro  
210 215 220  
Leu Glu Trp Pro Leu Ser Asn Gln Ser Gly Ser Tyr Glu Leu Arg Ile  
225 230 235 240  
Glu Val Gln Pro Lys Pro His His Arg Ala His Tyr Glu Thr Glu Gly  
245 250 255  
Ser Arg Gly Ala Val Lys Ala Pro Thr Gly Gly His Pro Val Val Gln  
260 265 270  
Leu His Gly Tyr Met Glu Asn Lys Pro Leu Gly Leu Gln Ile Phe Ile  
275 280 285  
Gly Thr Ala Asp Glu Arg Ile Leu Lys Pro His Ala Phe Tyr Gln Val  
290 295 300  
His Arg Ile Thr Gly Lys Thr Val Thr Thr Ser Tyr Glu Lys Ile  
305 310 315 320

7

Val Gly Asn Thr Lys Val Leu Glu Ile Pro Leu Glu Pro Lys Asn Asn  
325 330 335

Met Arg Ala Thr Ile Asp Cys Ala Gly Ile Leu Lys Leu Arg Asn Ala  
340 345 350

Asp Ile Glu Leu Arg Lys Gly Glu Thr Asp Ile Gly Arg Lys Asn Thr  
355 360 365

Arg Val Arg Leu Val Phe Arg Val His Val Pro Glu Pro Ser Gly Arg  
370 375 380

Ile Val Ser Leu Gln Ala Ala Ser Asn Pro Ile Glu Cys Ser Gln Arg  
385 390 395 400

Ser Ala His Glu Leu Pro Met Val Glu Arg Gln Asp Met Asp Ser Cys  
405 410 415

Leu Val Tyr Gly Gly Gln Met Ile Leu Thr Gly Gln Asn Phe Thr  
420 425 430

Ala Glu Ser Lys Val Val Phe Met Glu Lys Thr Thr Asp Gly Gln Gln  
435 440 445

Ile Trp Glu Met Glu Ala Thr Val Asp Lys Asp Lys Ser Gln Pro Asn  
450 455 460

Met Leu Phe Val Glu Ile Pro Glu Tyr Arg Asn Lys His Ile Arg Val  
465 470 475 480

Pro Val Lys Val Asn Phe Tyr Val Ile Asn Gly Lys Arg Lys Arg Ser  
485 490 495

Gln Pro Gln His Phe Thr Tyr His Pro Val Pro Ala Ile Lys Thr Glu  
500 505 510

Pro Ser Asp Glu Tyr Glu Pro Ser Leu Ile Cys Ser Pro Ala His Gly  
515 520 525

Gly Leu Gly Ser Gln Pro Tyr Tyr Pro Gln His Pro Met Leu Ala Glu  
530 535 540

Ser Pro Ser Cys Leu Val Ala Thr Met Ala Pro Cys Gln Gln Phe Arg  
545 550 555 560

Ser Gly Leu Ser Ser Pro Asp Ala Arg Tyr Gln Gln Ser Pro Ala  
565 570 575

Ala Ala Leu Tyr Gln Arg Ser Lys Ser Leu Ser Pro Gly Leu Leu Gly  
580 585 590

Tyr Gln Gln Pro Ser Leu Leu Ala Ala Pro Leu Gly Leu Ala Asp Ala  
595 600 605

His Arg Ser Val Leu Val His Ala Gly Ser Gln Gly Gln Gly Gln Gly  
610 615 620

Ser Thr Leu Arg His Thr Ser Ser Ala Ser Gln Gln Ala Ser Pro Val  
625 630 635 640

Ile His Tyr Ser Pro Thr Asn Gln Gln Leu Arg Gly Gly Gly His Gln  
645 650 655

Glu Phe Gln His Ile Met Tyr Cys Glu Asn Phe Gly Pro Ser Ser Ala  
660 665 670

8

Arg Pro Gly Pro Pro Pro Ile Asn Gln Gly Gln Arg Leu Ser Pro Gly  
 675. 680 685

Ala Tyr Pro Thr Val Ile Gln Gln Gln Thr Ala Pro Ser Gln Arg Ala  
 690 695 700

Ala Lys Asn Gly Pro Ser Asp Gln Lys Glu Ala Leu Pro Thr Gly Val  
 705 710 715 720

Thr Val Lys Gln Glu Gln Asn Leu Asp Gln Thr Tyr Leu Asp Asp Ala  
 725 730 735

Ala Thr Ser Glu Ser Trp Val Gly Thr Glu Arg Tyr Ile Glu Arg Lys  
 740 745 750

Phe Trp Lys Lys Thr Leu Val Gln Pro Gly Leu Leu Pro Ser Phe Leu  
 755 760 765

Leu Leu Gly Ser Leu Ser Ala Gly Pro Arg Ser Gln Thr Pro Ser Glu  
 770 775 780

Arg Lys Pro Ile Glu Glu Asp Val Pro Leu Ser Cys Ser Gln Ile Ala  
 785 790 795 800

Trp Cys Cys Gln His Pro Leu Gly Thr Cys Pro Val Leu Pro Gly Pro  
 805 810 815

Leu Ala Val Glu Trp Trp Glu Gly Gln Leu Gly Arg Gly Leu Glu Pro  
 820 825 830

Ile Pro Trp Ala Pro Asp Ser Ala Gly Ser Leu His Glu Val Asp Ser  
 835 840 845

Val Gly Leu Ala Gly Val Val Gly Met Val Leu Leu Thr Leu Met His  
 850 855 860

His Phe Ser Met Asp Gln Asn Gln Thr Pro Ser Pro His Trp Gln Arg  
 865 870 875 880

His Lys Glu Val Ala Ser Pro Gly Trp Ile  
 885 890

<210> 15  
<211> 29  
<212> DNA  
<213> Muridae sp.

<220>  
<223> DNA sequence binding pair: NF-AT site

<300>  
<310> 5,656,452  
<311> 1993-10-29  
<312> 1997-08-12

<400> 15  
gccccaaagag gaaaatttgtt ttcatacag

29

<210> 16  
<211> 160  
<212> PRT  
<213> Homo sapiens

&lt;220&gt;

9

&lt;223&gt; Human helix-loop-helix zipper protein DNA binding sequence: MAX

<300>  
<310> 5,693,487  
<311> 1994-04-01  
<312> 1997-12-02<400> 16  
Met Ser Asp Asn Asp Asp Ile Glu Val Glu Ser Asp Glu Glu Gln Pro  
1 5 10 15  
Arg Phe Gln Ser Ala Ala Asp Lys Arg Ala His His Asn Ala Leu Glu  
20 25 30  
Arg Lys Arg Arg Asp His Ile Lys Asp Ser Phe His Ser Leu Arg Asp  
35 40 45  
Ser Val Pro Ser Leu Gln Gly Glu Lys Ala Ser Arg Ala Gln Ile Leu  
50 55 60  
Asp Lys Ala Thr Glu Tyr Ile Gln Tyr Met Arg Arg Lys Asn His Thr  
65 70 75 80  
His Gln Gln Asp Ile Asp Asp Leu Lys Arg Gln Asn Ala Leu Leu Glu  
85 90 95  
Gln Gln Val Arg Ala Leu Glu Lys Ala Arg Ser Ser Ala Gln Leu Gln  
100 105 110  
Thr Asn Tyr Pro Ser Ser Asp Asn Ser Leu Tyr Thr Asn Ala Lys Gly  
115 120 125  
Ser Thr Ile Ser Ala Phe Asp Gly Gly Ser Asp Ser Ser Ser Glu Ser  
130 135 140  
Glu Pro Glu Glu Pro Gln Ser Arg Lys Lys Leu Arg Met Glu Ala Ser  
145 150 155 160<210> 17  
<211> 533  
<212> PRT  
<213> Homo sapiens<220>  
<223> Human Egr Dna binding protein: zinc finger domain<300>  
<310> 5,866,325  
<311> 1995-06-06  
<312> 1999-02-02<400> 17  
Met Ala Ala Ala Lys Ala Glu Met Gln Leu Met Ser Pro Leu Gln Ile  
1 5 10 15  
Ser Asp Pro Phe Gly Ser Phe Pro His Ser Pro Thr Met Asp Asn Tyr  
20 25 30  
Pro Lys Leu Glu Glu Met Met Leu Leu Ser Asn Gly Ala Pro Gln Phe  
35 40 45

10

Leu Gly Ala Ala Gly Thr Pro Glu Gly Ser Gly Gly Asn Ser Ser Ser  
50 55 60

Ser Thr Ser Ser Gly Gly Gly Gly Gly Ser Asn Ser Gly Ser  
65 70 75 80

Ser Ala Phe Asn Pro Gln Gly Glu Pro Ser Glu Gln Pro Tyr Glu His  
85 90 95

Leu Thr Thr Glu Ser Phe Ser Asp Ile Ala Leu Asn Asn Glu Lys Ala  
100 105 110

Met Val Glu Thr Ser Tyr Pro Ser Gln Thr Thr Arg Leu Pro Pro Ile  
115 120 125

Thr Tyr Thr Gly Arg Phe Ser Leu Glu Pro Ala Pro Asn Ser Gly Asn  
130 135 140

Thr Leu Trp Pro Glu Pro Leu Phe Ser Leu Val Ser Gly Leu Val Ser  
145 150 155 160

Met Thr Asn Pro Pro Thr Ser Ser Ser Ala Pro Ser Pro Ala Ala  
165 170 175

Ser Ser Ser Ser Ala Ser Gln Ser Pro Pro Leu Ser Cys Ala Val  
180 185 190

Pro Ser Asn Asp Ser Ser Pro Ile Tyr Ser Ala Ala Pro Thr Phe Pro  
195 200 205

Thr Pro Asn Thr Asp Ile Phe Pro Glu Pro Gln Ser Gln Ala Phe Pro  
210 215 220

Gly Ser Ala Gly Thr Ala Leu Gln Tyr Pro Pro Ala Tyr Pro Ala  
225 230 235 240

Thr Lys Gly Phe Gln Val Pro Met Ile Pro Asp Tyr Leu Phe Pro  
245 250 255

Gln Gln Gln Gly Asp Leu Ser Leu Gly Thr Pro Asp Gln Lys Pro Phe  
260 265 270

Gln Gly Leu Glu Asn Arg Thr Gln Gln Pro Ser Leu Thr Pro Leu Ser  
275 280 285

Thr Ile Lys Ala Phe Ala Thr Gln Ser Gly Ser Gln Asp Leu Lys Ala  
290 295 300

Leu Asn Thr Thr Tyr Gln Ser Gln Leu Ile Lys Pro Ser Arg Met Arg  
305 310 315 320

Lys Tyr Pro Asn Arg Pro Ser Lys Thr Pro Pro His Glu Arg Pro Tyr  
325 330 335

Ala Cys Pro Val Glu Ser Cys Asp Arg Arg Phe Ser Arg Ser Asp Glu  
340 345 350

Leu Thr Arg His Ile Arg Ile His Thr Gly Gln Lys Pro Phe Gln Cys  
355 360 365

Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp His Leu Thr Thr His  
370 375 380

Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys Asp Ile Cys Gly  
385 390 395 400

11

Arg Lys Phe Ala Arg Ser Asp Glu Arg Lys Arg His Thr Lys Ile His  
405 410 415

Leu Arg Gln Lys Asp Lys Lys Ala Asp Lys Ser Val Val Ala Ser Pro  
420 425 430

Ala Ala Ser Ser Leu Ser Ser Tyr Pro Ser Pro Val Ala Thr Ser Tyr  
435 440 445

Pro Ser Pro Ala Thr Thr Ser Phe Pro Ser Pro Val Pro Thr Ser Tyr  
450 455 460

Ser Ser Pro Gly Ser Ser Thr Tyr Pro Ser Pro Ala His Ser Gly Phe  
465 470 475 480

Pro Ser Pro Ser Val Ala Thr Thr Phe Ala Ser Val Pro Pro Ala Phe  
485 490 495

Pro Thr Gln Val Ser Ser Phe Pro Ser Ala Gly Val Ser Ser Phe  
500 505 510

Ser Thr Ser Thr Gly Leu Ser Asp Met Thr Ala Thr Phe Ser Pro Arg  
515 520 525

Thr Ile Glu Ile Cys  
530

<210> 18  
<211> 224  
<212> PRT  
<213> Homo sapiens

<220>  
<223> Human S 1-3 DNA binding protein: zinc finger domain

<300>  
<310> 5,905,146  
<311> 1996-03-15  
<312> 1999-05-18

<400> 18  
Pro Ile Glu Val Cys Arg Ser Lys Leu Ser Lys Tyr Leu Gln Gly Val  
1 5 10 15

Val Phe Arg Cys Asp Lys Cys Thr Phe Thr Cys Ser Ser Asp Glu Ser  
20 25 30

Leu Gln Gln His Ile Glu Lys His Asn Glu Leu Lys Pro Tyr Lys Cys  
35 40 45

Gln Leu Cys Tyr Tyr Glu Thr Lys His Thr Glu Glu Leu Asp Ser His  
50 55 60

Leu Arg Asn Glu His Lys Val Ser Arg Asn Phe Glu Leu Val Gly Arg  
65 70 75 80

Val Asn Leu Asp Gln Leu Glu Gln Met Lys Glu Lys Met Glu Ser Ser  
85 90 95

Ser Ser Asp Asp Glu Asp Lys Glu Glu Met Asn Ser Lys Ala Glu  
100 105 110

Asp Arg Glu Leu Met Arg Phe Ser Asp His Gly Ala Ala Leu Asn Thr  
115 120 125

12

Glu Lys Arg Phe Pro Cys Glu Phe Cys Gly Arg Ala Phe Ser Gln Ala  
 130 135 140

Ser Glu Trp Glu Arg His Val Leu Arg His Gly Met Ala Leu Asn Asp  
 145 150 155 160

Thr Lys Gln Val Ser Arg Glu Ile His Pro Lys Glu Ile Met Glu  
 165 170 175

Asn Ser Val Lys Met Pro Ser Ile Glu Glu Lys Glu Asp Asp Glu Ala  
 180 185 190

Ile Gly Ile Asp Phe Ser Leu Lys Asn Glu Thr Val Ala Ile Cys Val  
 195 200 205

Val Thr Ala Asp Lys Ser Leu Leu Glu Asn Ala Glu Ala Lys Lys Glu  
 210 215 220

<210> 19  
<211> 272  
<212> RNA  
<213> Rattus sp.

<220>  
<223> Untranslated region (UTR) of rat glucose transporter mRNA (GLUT1)

<300>  
<310> 5,859,227  
<311> 1997-02-20  
<312> 1999-01-12

<400> 19  
ggggcgggccc aauggcggcg guccuaauaa aaggcagcuc cgcgcgcucu cuuccuaaga 60  
acacaagaau cccuugugga gugucgguuu agguugcagg gucuaaagug agucagggcg 120  
cgaggguccg gcgggagacg cauagucaca gaacguccau ucuccguuuc acagccccca 180  
cageuugagc cucgagcgca ggcggccau ggagcccgac agcaagaagg ugacgggccc 240  
ccuuaugug gccgugggag gggcagugcu cg 272

<210> 20  
<211> 108  
<212> RNA  
<213> Homo sapiens

<220>  
<223> 5' untranslated region (UTR) of human  
 3-hydroxy-3-methyl-3-glutaryl CoA reductase  
 (HMG,CoA Red)

<300>  
<310> 5,859,227  
<311> 1997-02-20  
<312> 1999-01-12

<400> 20  
uccuuccgcu cgcgcacugc guuaacugga gccaggcuca ggcgcggcgc cgggguucgg 60  
uggccucuag ugagaucugg aggauccaaag gauucuguag cuacaaug 108

<210> 21  
<211> 107  
<212> RNA  
<213> Homo sapiens

<220>  
<223> 5' untranslated region (UTR) of human C4b-binding protein- alpha chain

<300>  
<310> 5,859,227  
<311> 1997-02-20  
<312> 1999-01-12

<400> 21  
gcugcuuau uucugcuguu aaucauucau ugggcccgcuc aaaaguuuucu gcccaucuaau 60  
uuccauaaca cguccuugac cagccaaacca cauggcugaa auucagg 107

<210> 22  
<211> 174  
<212> RNA  
<213> Homo sapiens

<220>  
<223> 5' untranslated region (UTR) of human CD45

<300>  
<310> 5,859,227  
<311> 1997-02-20  
<312> 1999-01-12

<400> 22  
cugacaucau caccuagcag mucaugcagc uagcaagugg uuuguuucua ggguaacaga 60  
ggaggaaauu guuccucguc ugauaagaca acaguggaga aaggacgcau gcaguuucuu 120  
agggacacgg cugacuucca gauaugacca uguauuugug gcuuaaacuc wugg 174

<210> 23  
<211> 25  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Peptide G25  
representing the putative lipid-binding region (G region) of the erythrocyte Ca<sup>2+</sup> pump

<400> 23  
Lys Lys Ala Val Lys Val Pro Lys Lys Glu Lys Ser Val Leu Gln Gly  
1 5 10 15  
Lys Leu Thr Arg Leu Ala Val Gln Ile  
20 25

<210> 24  
<211> 39  
<212> DNA  
<213> Artificial Sequence

<220>

14

<223> Description of Artificial Sequence: Primer  
oligonucleotide

<220>  
<223> The NNS triplet degeneracy is repeated depending  
on the desired length of the complementarity  
determining region (CDR).

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 24  
gtgtattatt gtgcgagann stggggccaa gggaccacg

39

<210> 25  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide representing metal-binding protein surface  
domains from human histidine rich glycoprotein  
(HRG).

<300>  
<303> J. Chromatogr.  
<304> 604  
<305> 1  
<306> 125-132  
<307> 1992

<400> 25  
Gly His His Pro His  
1 5

<210> 26  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Mg (II) ion  
binding protein sequence

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 26  
Ser Arg Arg Ser Arg His His Pro Arg Met Trp Asn Gly Leu Asp Val  
1 5 10 15

<210> 27  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Mg (II) ion

15

binding protein sequence.

&lt;300&gt;

<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

&lt;400&gt; 27

Gly Arg Phe Lys Arg Val Arg Asp Arg Trp Val Val Ile Phe Asp Phe  
1 5 10 15

&lt;210&gt; 28

<211> 16  
<212> PRT  
<213> Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Mg (II) ion  
binding protein sequence.

&lt;300&gt;

<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

&lt;400&gt; 28

Gly Val Ala Arg Ser Lys Lys Met Arg Gly Leu Trp Arg Leu Asp Val  
1 5 10 15

&lt;210&gt; 29

<211> 16  
<212> PRT  
<213> Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Mg (II) ion  
binding protein sequence.

&lt;300&gt;

<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

&lt;400&gt; 29

Gly Leu Ala Val Arg Ser Lys Arg Gly Arg Phe Phe Leu Phe Asp Val  
1 5 10 15

&lt;210&gt; 30

<211> 10  
<212> PRT  
<213> Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: CU (II) ion  
binding protein sequence.

&lt;300&gt;

<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

&lt;400&gt; 30

Gly Arg Val His His His Ser Leu Asp Val

1                   5                   10                   16

<210> 31  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence : CU (II) ion binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 31  
Ser Trp Lys His His Ala His Trp Asp Val  
1                   5                   10

<210> 32  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Cu (II) ion binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 32  
Gly Ser Trp Asp His Arg Gly Cys Asp Gly  
1                   5                   10

<210> 33  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Cu (II) ion binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 33  
Gly His His Met Tyr Gly Gly Trp Asp His  
1                   5                   10

<210> 34  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>

17

<223> Description of Artificial Sequence: Cu (II) ion binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 34  
Gly His Trp Gly Arg His Ser Leu Asp Thr  
1 5 10

<210> 35  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Cu (II) ion binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 35  
Gly His Ile Leu His His Gln Leu Asp Leu  
1 5 10

<210> 36  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Cu (II) ion binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 36  
Ser Ser Gln Arg Leu Met Leu Gly Asp Asn  
1 5 10

<210> 37  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Cu (II) ion binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 37

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Ser His His Gly His His Tyr Leu Asn His  
1 5 10

<210> 38  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Cu (II) ion  
binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 38  
Gly Lys Leu Met Met Ser Trp Cys Arg Asp Thr Glu Gly Cys Asp His  
1 5 10 15

<210> 39  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Cu (II) ion  
binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 39  
Gly Asp Thr His Arg Gly His Leu Arg His His Leu Pro His Asp Trp  
1 5 10 15

<210> 40  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Cu (II) ion  
binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 40  
Gly Trp Gly Leu Trp Met Lys Pro Phe Val Trp Arg Ala Trp Asp Met  
1 5 10 15

<210> 41  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>

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<223> Description of Artificial Sequence: Zn (II) ion  
binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 41  
Gly Arg Val His His His Ser Leu Asp Val  
1 5 10

<210> 42  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Zn (II) ion  
binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 42  
Ser His Thr His Ala Leu Pro Leu Asp Phe  
1 5 10

<210> 43  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Zn (II) ion  
binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 43  
Gly Gln Ser Ser Gly Gly Asp Thr Asp Asp  
1 5 10

<210> 44  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Zn (II) ion  
binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

20

<400> 44  
Gly Gln Trp Thr Pro Arg Gly Asp Asp Phe  
1 5 10

<210> 45  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Zn (II) ion  
binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 45  
Gly Arg Cys Cys Pro Ser Ser Cys Asp Glu  
1 5 10

<210> 46  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Zn (II) ion  
binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 46  
Gly Pro Ala Lys His Arg His Arg His Val Gly Gln Met His Asp Ser  
1 5 10 15

<210> 47  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Pb (III) ion  
binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 47  
Gly Asn Leu Arg Arg Lys Thr Ser Asp Ile  
1 5 10

<210> 48  
<211> 10  
<212> PRT

21

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Pb (III) ion  
binding protein sequence.

&lt;300&gt;

&lt;310&gt; 5,679,548

&lt;311&gt; 1993-06-14

&lt;312&gt; 1997-10-21

&lt;400&gt; 48

Gly Glu Ser Asp Ser Lys Arg Glu Asp Gly  
1 5 10

&lt;210&gt; 49

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Pb (III) ion  
binding protein sequence.

&lt;300&gt;

&lt;310&gt; 5,679,548

&lt;311&gt; 1993-06-14

&lt;312&gt; 1997-10-21

&lt;400&gt; 49

Gly Gly Pro Ser Leu Ala Val Gly Asp Trp  
1 5 10

&lt;210&gt; 50

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Pb (III) ion  
binding protein sequence.

&lt;300&gt;

&lt;310&gt; 5,679,548

&lt;311&gt; 1993-06-14

&lt;312&gt; 1997-10-21

&lt;400&gt; 50

Gly Pro Leu Gln His Thr Tyr Pro Asp Tyr  
1 5 10

&lt;210&gt; 51

&lt;211&gt; 16

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Pb (III) ion  
binding protein sequence.

&lt;300&gt;

&lt;310&gt; 5,679,548

&lt;311&gt; 1993-06-14

22

&lt;312&gt; 1997-10-21

<400> 51  
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1 5 10 15

<210> 52  
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<220>  
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binding protein sequence.

<300>  
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<311> 1993-06-14  
<312> 1997-10-21

<400> 52  
Gly Thr Arg Val Trp Arg Val Cys Gln Trp Asn His Glu Glu Asp Gly  
1 5 10 15

<210> 53  
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<220>  
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<300>  
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<311> 1993-06-14  
<312> 1997-10-21

<400> 53  
Gly Glu Trp Trp Cys Ser Phe Ala Met Cys Pro Ala Arg Trp Asp Phe  
1 5 10 15

<210> 54  
<211> 16  
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<300>  
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<311> 1993-06-14  
<312> 1997-10-21

<400> 54  
Gly Asp Thr Ile Phe Gly Val Thr Met Gly Tyr Tyr Ala Met Asp Val  
1 5 10 15

<210> 55  
<211> 10

23

<212> PRT  
<213> Artificial Sequence

<220>  
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<300>  
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<311> 1993-06-14  
<312> 1997-10-21

<400> 55  
Gly Gln Val Met Gln Glu Leu Gly Asp Ala  
1 5 10

<210> 56  
<211> 10  
<212> PRT  
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<220>  
<223> Description of Artificial Sequence: Ce (III) ion  
binding protein sequence.

<300>  
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<311> 1993-06-14  
<312> 1997-10-21

<400> 56  
Gly Leu Thr Glu Gln Gln Leu Gln Asp Gly  
1 5 10

<210> 57  
<211> 10  
<212> PRT  
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<220>  
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<300>  
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<400> 57  
Gly Tyr Ser Tyr Ser Val Ser Pro Asp Ala  
1 5 10

<210> 58  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Ce (III) ion  
binding protein sequence.

<300>  
<310> 5,679,548

24

<311> 1993-06-14  
<312> 1997-10-21

<400> 58  
Gly Arg Leu Gly Leu Val Met Thr Asp Glu  
1 5 10

<210> 59  
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<212> PRT  
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<220>  
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binding protein sequence.

<300>  
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<312> 1997-10-21

<400> 59  
Ser Thr Trp Pro Gly Arg Gln Arg Leu Gly Gln Ala Leu Ser Asp Ser  
1 5 10 15

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<212> PRT  
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<300>  
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<400> 60  
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binding protein sequence.

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Gly Pro Val Arg Gly Leu Asp Gln Ser Lys Gly Val Arg Tyr Asp Asn  
1 5 10 15

<210> 62

25

<211> 16  
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binding protein sequence.

<300>  
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<312> 1997-10-21

<400> 62  
Gly Leu Ser Gln His Ile Val Ser Glu Thr Gln Ser Ser Gly Asp Leu  
1 5 10 15

<210> 63  
<211> 16  
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binding protein sequence.

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<312> 1997-10-21

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1 5 10 15

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<212> PRT  
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binding protein sequence.

<300>  
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<311> 1993-06-14  
<312> 1997-10-21

<400> 64  
Gly Asn Met Ile Leu Gly Gly Pro Gly Cys Trp Ser Ser Ala Asp Ile  
1 5 10 15

<210> 65  
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<311> 1993-06-14  
<312> 1997-10-21

<400> 65  
Gly Cys Trp Asn Val Gln Arg Leu Val Val Tyr His Pro Pro Asp Gly  
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<210> 66  
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<220>  
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binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 66  
Gly Phe Glu Val Thr Cys Ser Trp Phe Gly His Trp Gly Arg Asp Ser  
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<210> 67  
<211> 16  
<212> PRT  
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binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 67  
Ser Ala Ser Met Arg Ser Ala Ile Gly Leu Trp Arg Thr Met Asp Tyr  
1 5 10 15

<210> 68  
<211> 16  
<212> PRT  
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<220>  
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binding protein sequence.

<300>  
<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

<400> 68  
Gly Asp Arg Glu Ile Phe His Met Gln Trp Pro Leu Arg Val Asp Val  
1 5 10 15

<210> 69  
<211> 16  
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binding protein sequence.

<300>  
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<311> 1993-06-14  
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<400> 69  
Ser Gln Asn Pro Gln Gln Val Cys Gly Val Arg Cys Gly Gln Asp Lys  
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binding protein sequence.

<300>  
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<311> 1993-06-14  
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<400> 70  
Gly Asn Arg Leu Ser Ser Gly His Leu Leu Lys Gln Gly Gln Asp Gly  
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<210> 71  
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binding protein sequence.

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Gly Gly Ser Asp Trp Gln Ile Gly Ala Cys Cys Arg Glu Asp Asp Leu  
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<210> 72  
<211> 16  
<212> PRT  
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<220>  
<223> Description of Artificial Sequence: Fe (III) ion  
binding protein sequence.

28

&lt;300&gt;

<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

&lt;400&gt; 72

Gly Met Val Ser Met Met Gly Gln Ser Arg Pro Thr Gln Cys Asp Cys  
1 5 10 15

&lt;210&gt; 73

&lt;211&gt; 16

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Fe (III) ion  
binding protein sequence.

&lt;300&gt;

<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

&lt;400&gt; 73

Gly Val Ile Lys Trp Ile Arg Arg Trp Val Arg Thr Ala Arg Asp Val  
1 5 10 15

&lt;210&gt; 74

&lt;211&gt; 16

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Fe (III) ion  
binding protein sequence.

&lt;300&gt;

<310> 5,679,548  
<311> 1993-06-14  
<312> 1997-10-21

&lt;400&gt; 74

Gly Trp Phe Trp Arg Leu Leu Pro Thr Pro Arg Ala Pro Ser Asp Val  
1 5 10 15

&lt;210&gt; 75

<211> 90<sup>9</sup>

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (74) .. (541)

&lt;220&gt;

&lt;223&gt; Human glutathione S-transferase cDNA

&lt;300&gt;

&lt;308&gt; J03746/GenBank

&lt;400&gt; 75

gaattcaagt cctaaaggct acagtttga atactactga aatgacaagt tattccagac 50

29

caaaattgaa aaa atg gtt gac ctc acc cag gta atg gat gat gaa gta 109  
 Met Val Asp Ieu Thr Gln Val Met Asp Asp Glu Val  
 1 5 10

tcc atg gct ttt gca tcc tat gca aca att att ctt tca aaa atg atg 157  
 Phe Met Ala Phe Ala Ser Tyr Ala Thr Ile Ile Leu Ser Lys Met Met  
 15 20 25

ctt atg agt act gca act gca ttc tat aga ttg aca aga aag gtt ttt 205  
 Leu Met Ser Thr Ala Thr Ala Phe Tyr Arg Leu Thr Arg Lys Val Phe  
 30 35 40

gcc aat cca gaa gac tgt gta gca ttt ggc aaa gga gaa aat gcc aag 253  
 Ala Asn Pro Glu Asp Cys Val Ala Phe Gly Lys Gly Glu Asn Ala Lys  
 45 50 60

aag tat ctt cga aca gat gac aga gta gaa cgt gta cgc aga gcc cac 301  
 Lys Tyr Leu Arg Thr Asp Asp Arg Val Glu Arg Val Arg Arg Ala His  
 65 70 75

ctg aat gac ctt gaa aat att att cca ttt ctt gga att ggc ctc ctg 349  
 Leu Asn Asp Leu Glu Asn Ile Ile Pro Phe Leu Gly Ile Gly Leu Leu  
 80 85 90

tat tcc ttg agt ggt ccc gac ccc tct aca gcc atc ctg cac ttc aga 397  
 Tyr Ser Leu Ser Gly Pro Asp Pro Ser Thr Ala Ile Leu His Phe Arg  
 95 100 105

cta ttt gtc gga gca cgg atc tac cac acc att gca tat ttg aca ccc 445  
 Leu Phe Val Gly Ala Arg Ile Tyr His Thr Ile Ala Tyr Leu Thr Pro  
 110 115 120

ctt ccc cag cca aat aga gct ttg agt ttt ttt gtt gga tat gga gtt 493  
 Leu Pro Gln Pro Asn Arg Ala Leu Ser Phe Phe Val Gly Tyr Gly Val  
 125 130 135 140

act ctt tcc atg gct tac agg ttg ctg aaa agt aaa ttg tac ctg taa 541  
 Thr Leu Ser Met Ala Tyr Arg Leu Leu Lys Ser Lys Leu Tyr Leu  
 145 150 155

agaaaatcat acaaactcaac atccagttgg ctttttaaga attctgtact tccaaatttat 601  
 aatgaatact ttcttagatt ttaggttagga ggggagcaga ggaattatga actggggtaa 661  
 acccattttg aatatttagca ttgccaatat cctgtattct tgttttacat ttggatttaga 721  
 aatttaacat agtaattctt aagtcttttgc tctgatttt aaagtaacttt ctatataatt 781  
 tggatcatgt tatgatttgt aacattcaca caacacctca cttttgaatc tataaaagaa 841  
 ttgcacgtat gagaaaccta tatttcaata ctgctgaaac agacatgaaa taaagaattt 901  
 aaagaatg 909

&lt;210&gt; 76

&lt;211&gt; 155

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 76

Met Val Asp Leu Thr Gln Val Met Asp Asp Glu Val Phe Met Ala Phe  
 1 5 10 15

Ala Ser Tyr Ala Thr Ile Ile Leu Ser Lys Met Met Leu Met Ser Thr  
 20 25 30

30

Ala Thr Ala Phe Tyr Arg Leu Thr Arg Lys Val Phe Ala Asn Pro Glu  
35 40 45

Asp Cys Val Ala Phe Gly Lys Gly Glu Asn Ala Lys Lys Tyr Leu Arg  
50 55 60

Thr Asp Asp Arg Val Glu Arg Val Arg Arg Ala His Leu Asn Asp Leu  
65 70 75 80

Glu Asn Ile Ile Pro Phe Leu Gly Ile Gly Leu Leu Tyr Ser Leu Ser  
85 90 95

Gly Pro Asp Pro Ser Thr Ala Ile Leu His Phe Arg Leu Phe Val Gly  
100 105 110

Ala Arg Ile Tyr His Thr Ile Ala Tyr Leu Thr Pro Leu Pro Gln Pro  
115 120 125

Asn Arg Ala Leu Ser Phe Phe Val Gly Tyr Gly Val Thr Leu Ser Met  
130 135 140

Ala Tyr Arg Leu Leu Lys Ser Lys Leu Tyr Leu  
145 150 155

<210> 77  
<211> 4

<212> PRT  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Tetrapeptide  
prior to cleavage site of Factor Xa recognition  
sequence.

<300>

<303> Nature  
<304> 309  
<305> 810  
<307> 1984

<400> 77

Ile Glu Gly Arg  
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<210> 78

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PreScission  
Protease recognition sequence.

<220>

<223> PreScission Protease cleaves between Gln and Gly.

<300>

<303> J. Biol. Chem.  
<304> 265

<306> 9062

<307> 1990

<400> 78

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PCT/US01/00452

31

Leu Glu Val Leu Phe Gln Gly Pro  
1 5